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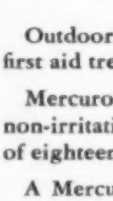

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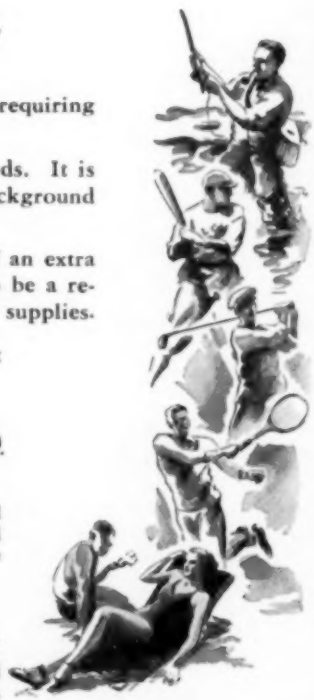

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Studies on Colloidal Sulfur—Polysulphide Mixture. I.—Toxicity*

By Harry Greengard, Ph.D., M.D., and Jean Rea Woolley, M.D.

INTRODUCTION

An investigation has been made of a colloidal sulfur preparation which is a concentrate of a naturally occurring spring water[1] and differs from the native water mainly in that it is quite alkaline (p_H of 10.1) and contains a large amount of sulfur (5 per cent). Much of the sulfur is in the colloidal state, since the Tyndall phenomenon is demonstrable, and due to the alkalinity of the solution, there is also some polysulphide present. The proportion of polysulphide sulfur cannot be determined, and there is reason to believe that for practical purposes it is equivalent to colloidal sulfur. The solution is transparent, orange-red in color, has a very pronounced sulfurous odor and taste, and undergoes precipitation on acidification or on heating to above 90° C.

The activity of sulfur in the organism appears to be a function of the degree to which it may be converted to H_2S. Various investigators (1–6) have demonstrated that such a reaction occurs when living organic matter acts on sulfur; hence it follows that the toxic manifestations of sulfur absorption are those of sulphide intoxication. The stigmata of such intoxication have been reported (7), (8) to be narcosis, convulsions, central motor depression, pulmonary edema and hyperemia and cardiac irregularities with terminal arrest; and several workers (9–15) have noted that the results when colloidal sulfur was given in toxic doses were identical with H_2S or sulphide poisoning. The more recent work (13), (14) has shown that the degree of toxicity of colloidal sulfur depends upon the route of administration, the degree of dispersion of the colloidal particles and the speed of injection when the material is given intravenously. The ability of the organism to survive a given dose of sulfur depends, therefore, on whether the resultant H_2S can be cleared from the system before it accumulates to a fatal level.

Toxic manifestations from sulfur administration have been reported clinically. Burmeister (16) described a case of violent poisoning after use of a sulfur-containing salve for scabies, evidenced by a marked burning sensation of the skin, followed by shock and syncope with recovery after

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[1] The source of this water is Graham Springs, Harrodsburg, Kentucky.

vigorous stimulation. Hesse (17) noted intoxications and febrile reactions occasionally when cases of scabies or pediculosis were treated with sulfur. Basch (18) had several fatalities in infants treated for scabies with a 10 per cent sulfur ointment; the autopsy findings were those of chronic H_2S intoxication. He stated that the absorbability of sulfur in this form depended on the degree of injury to the skin and that the intact skin is almost impervious, and he was able to duplicate this intoxication experimentally in animals (19).

In the present work, the toxicity of the preparation under investigation was studied with regard to its acute and chronic effects by various routes of administration. The minimal lethal dose was determined by the intravenous route in dogs, rats and rabbits; by the intraperitoneal route in guinea pigs; and by the oral route in rabbits. The effect on circulation and respiration of the anesthetized dog was studied. Finally, the material was given orally to a series of rats, and orally and intravenously to rabbits, with autopsy studies after an appropriate interval. It was also given to human subjects in large doses for seven days, after completion of the animal experiments.

EXPERIMENTAL

Minimal Lethal Dose Determinations.—In the light of the knowledge gained by other investigators regarding the effect of rate of injection on the toxicity of the material, it was considered necessary to standardize the intravenous injection rate for all species used. Therefore, the solution was diluted with distilled water to a concentration of 0.5 per cent and the injection was made over a period of exactly one minute at as constant a rate as possible. The rats were prepared by ether anesthesia and exposure of the external jugular vein through a small incision; after injection the wound was closed and recovery allowed to take place.

Effect on Circulation and Respiration in the Anesthetized Dog.—The dogs were anesthetized with sodium pentobarbital and tracings were made of carotid blood pressure and respiration in the usual manner. Gross and microscopic examinations of the lungs were made after death of the animals.

Chronic Toxicity.—This problem was investigated on rabbits and rats. Six rabbits were given a daily ration of 10 Gm. of alfalfa hay, 10 Gm. of sucrose and 150 cc. of milk. Three of them received, respectively, 0.5, 1.0 and 2.0 mg. per Kg. of colloidal sulfur daily intravenously, and the others 3–5, 10 and 20 mg. per Kg. per day orally. After a period of 20 days they were sacrificed and pathological studies were made of the kidneys, livers, stomach and lungs. The sulfur excretion of these animals was followed daily, and the number of analyses required made it impossible to use a larger series.

The rats were given a diet of the following composition:

Casein—18%
Dextrin—37%
Sucrose—15%
Salt mixture (Osborne-Mendel)—4%
Agar—2%
Cod liver oil—5%
Lard—19%

This diet and water were given *ad lib.* and supplemented with 400 mg. of yeast daily in the form of pills. After a preliminary 20-day period to permit the animals to demonstrate their ability to grow normally, daily doses of 2, 5, 10 and 15 mg. of colloidal sulfur were given; the sulfur was incorporated into the pill, and the animals maintained on this régime for 100 days.

Six human subjects were given the material daily for 10 days in a dosage of 500 to 750 mg. per individual, or 10 to 15 mg. per Kg. of body weight.

RESULTS

1. Minimal Lethal Dose Determinations.—The results are tabulated below.

Table I.—Minimum Lethal Dose

Species	Route of Administration	Dosage mg./Kg.	Lived	Died
Dog	Intravenous	10	16	14
Rabbit	Intravenous	5	8	11
Rat	Intravenous	8	12	9
Guinea pig	Intraperitoneal	55	10	12
Rabbit	Oral	175	12	13

Convulsions, syncope and an H_2S odor of the breath were the manifestations noted.

2. Effect on Circulation and Respiration.—There was uniformly a fall in blood pressure immediately after injection with bradycardia and an initial stimulation of respiration followed by arrest; the severity of these manifestations depended on the amount of material injected and on the rate of injection. There was invariably an accumulation of serous fluid in air passages, so marked when the dose was large as to pour out of the trachea in large amounts. Examination of the lungs revealed them to be boggy and very hemorrhagic; the bronchial tree was filled with a frothy fluid; and microscopically there was evident a pronounced edema of the alveolar walls. In order to establish that these phenomena were due to the sulfur some of the material was desulfurized by acidification and filtration followed by alkalinization to the original p_H. Injection of the desulfurized material in large amounts was without effect on the animals.

3. Chronic Toxicity.—Autopsy of the three rabbits which were given colloidal sulfur intra-

venously revealed thickening and fibrosis of the alveolar walls of the lungs. There were no abnormalities in the kidneys, livers or stomachs. In the case of the rabbits receiving the material orally in much larger doses there were no pathological changes in any of the organs.

The series of rats which were given sulfur at various levels for 120 days grew normally (Table II) and on autopsy no abnormalities were found. A second series of rats were also studied. At maturity these rats were mated, and it was found that both control and sulfur-fed rats gave birth to healthy litters which they nursed successfully.

The human subjects given the sulfur solution reported no unpleasant subjective symptoms from the ingestion.

Table II.—Effect of Oral Administration

Rat No.	Sulfur Daily	Initial Wt.	Final Wt.	Average Daily Gain	Average Daily Food Consumption
1	Control	40	245	1.7	7.8
2	Control	37	267	1.9	7.5
3	2 mg.	37	180	1.2	7.5
4	2 mg.	44	187	1.2	7.3
5	2 mg.	32	181	1.2	7.3
6	2 mg.	47	252	1.7	8.3
7	5 mg.	61	183	1.0	8.0
8	5 mg.	49	238	1.6	8.3
10	5 mg.	42	173	1.1	7.8
11	10 mg.	43	232	1.5	8.0
12	10 mg.	37	164	1.0	6.8
13	10 mg.	27	175	1.2	7.0
14	10 mg.	39	269	1.9	9.0
15	15 mg.	36	195	1.3	7.8
16	15 mg.	39	270	1.9	8.5
17	15 mg.	36	142	0.9	6.0
18	15 mg.	28	289	2.2	8.8
19	5 mg.	51	303	2.1	9.3

DISCUSSION

The findings reported above confirm the evidence in the literature to the effect that colloidal sulfur is highly toxic when given intravenously and that it owes its toxicity to rapid conversion to hydrogen sulphide. This gas is excreted for the most part, if not entirely, through the expired air; and in its passage through the lung it produces inflammatory changes as reflected acutely by hemorrhage and edema and chronically by fibrosis. The work of Wheeldon and Main (14) was also confirmed in that the material was much less toxic when injected intraperitoneally in guinea pigs, the M. L. D. as determined by them with the technic was 40 mg. per Kg. whereas in the product used in this work it was 55 mg. per Kg. It is obvious that the absorption of H_2S into the circulation is much slower in this case, so that considerably larger amounts may be tolerated.

No definite evidence exists in the literature that colloidal sulfur is toxic when given orally; and it was found in this work that 35 times the intravenous minimal lethal dose was tolerated without the production of deleterious effects by half of the animals to whom it was given by stomach tube. This may be explained either by the fact that the sulfur undergoes precipitation when it comes in contact with the acid gastric contents and is thereby rendered inert, or else that it is competely absorbed and so modified in its passage through the liver as to be rendered nontoxic unless the dose is overwhelming. Evidence given in another paper (20) indicates the latter view to be the correct one. The effect of oral sulfur administration continued over long periods of time in the rats, some of which received over 100 mg. per Kg. per day for over three months, shows that colloidal sulfur when given orally is in no way incompatible with normal growth and function. The administration of colloidal sulfur is not incompatible with pregnancy and lactation in the rat.

CONCLUSION

A colloidal sulfur-polysulphide preparation has been tested for toxicity by the intravenous, intraperitoneal and oral routes of administration. It was highly toxic when given intravenously and yielded an M. L. D. of 5 to 10 mg. of sulfur per Kg. of body weight, depending on the species used, when injection is made in one minute's time. The toxicity depends on the prompt conversion of the sulfur to H_2S, and death takes place from H_2S poisoning. Pathological findings in the lungs are edema and hemorrhage acutely, and fibrous tissue proliferation chronically. The same acute symptoms are noted with toxic doses by the intraperitoneal and oral routes, but much larger amounts are tolerated here, especially in the case of oral administration. Unless the dose is extreme, large amounts may be taken by mouth over a long period of time. The human subject easily tolerates up to 750 mg. of colloidal sulfur daily when given orally.

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Use of Sodium Pentobarbital for Repeated Anesthesia in the Rabbit

*By V. Everett Kinsey**

The need in this laboratory for an anesthetic which could be safely administered to small laboratory animals over periods of approximately one year gave rise to the present investigation. It was desired to find an anesthetic which could be easily given, required no further attention, and especially one which would be relatively safe and leave the animal in an apparently normal physiological state even though it be repeated every other day over a period of months.

Of the many anesthetics available it appeared that one of the medium to short-acting barbiturate derivatives would come nearest to meeting all of the above requirements. Many previous studies on the action of this series of compounds have been concerned with detoxification, elimination, the effect of single doses on various organs of the body and, particularly, with the hypnotic and minimal lethal dose. Tatum (1) has written a review of the status of the whole barbiturate problem. Investigations dealing with repeated administration of various barbiturates are adequately referred to here, by Masuda, *et al.* (2), and by others, some of whose papers will be discussed in more detail later. Most workers concur in the opinion that several barbiturates produce appreciable tolerance after administration for relatively short periods of time (days, not weeks) as measured by a lessening of sleeping time. Few of the workers, however, have subjected the animals to weeks or months of treatment and little stress has been placed on the important question of survival, either at the time of injection or later, although deaths presumably due directly to the anesthetic are recorded in several instances.

The choice of barbiturate appeared to be limited to those compounds which would produce sleep in small laboratory animals for periods not greatly in excess of two hours when given every other day. Several preliminary experiments confirmed this and showed that when, after repeated anesthesia, animals were kept unconscious even for two hours following a single injection of the barbiturate, long periods of depression followed. The debilitating effect of the latter apparently led to the extraordinarily high mortality which was observed. Because of these discouraging results, a shorter acting compound, sodium pentobarbital (Nembutal)[1] was chosen for this investigation, even though two or sometimes more separate injections were required to produce anesthesia for two-hour periods.

It is the purpose of the present study to review the whole question of repeated intraperitoneal injections of sodium pentobarbital in the rabbit from the viewpoint of the in-

* Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

[1] Kindly furnished by the Abbott Laboratories, North Chicago, Ill.

vestigator who has need of frequent recurring anesthesia. Particular emphasis will be placed on such practical considerations as dosage, tolerance (as evidenced by reduced sleeping time) and mortality.

MATERIALS AND METHODS

Powdered sodium pentobarbital was weighed out accurately at intervals and dissolved in sterile salt solution (0.9 per cent NaCl) containing 10 per cent ethyl alcohol[2] in concentrations of 65 mg./ml. In this medium the Nembutal stayed permanently in solution and when kept at room temperature in bottles stoppered with rubber diaphragm caps, remained sterile with ordinary precautions over the period of time needed to use all of the particular lot—usually about a week.

Every injection was given intraperitoneally with a one-half inch gage 27 needle. It is likely that an occasional dose would be injected subcutaneously or possibly into the rather thick scar tissue which eventually formed in most of the animals, and no doubt, too, now and then, the intestine would be punctured, although no known peritoneal infection resulted from the latter. In view of these possibilities, the occasional animal which would fail to go to sleep on one day was disregarded in figuring the average sleeping times.

The abdomens of the rabbits were clipped periodically, and the site of the injection was thoroughly wet with 70 per cent alcohol before administering the anesthetic. Only two local infections were found and these may have resulted from the bites of other rabbits. All of the animals were weighed before every injection.

Two criteria were used to measure the sleeping time of the rabbits: (*a*) the time during which an animal would not move when infrared radiations were applied to the eye,[3] and (*b*) the time during which the righting reflex was lost. Although many comparative tests showed that the sleeping times as judged by the two criteria were the same, the first criterion only was used in judging groups A, B and C while the second was used for all of the other rabbits considered.

EXPERIMENTAL

The investigations with rabbits involved two essentially separate problems. The first was concerned with the effect on the sleeping time of repeated injections of a particular dose of Nembutal, while the second dealt with the dose of Nembutal which would be necessary to produce a particular sleeping time.

About equal numbers of healthy young, adult Dutch and Chinchilla rabbits (no albinos) were selected from stock and acclimated to life in a small well-ventilated animal room where they were kept in fairly large individual wire bottom cages. They were fed alfalfa hay every morning, a cupful of soaked oats and bran mixture during the late afternoon and carefully selected greens between two and four times weekly. That the animal quarters and diet were quite adequate is evidenced from the following: (*a*) the average weight of the rabbits increased from 2300 to approximately 2700 Gm., notwithstanding the repeated anesthesias to which they were subjected; (*b*) the mortality, other than those animals dying at the time of anesthesia or killed deliberately, was less than 10 per cent for a year and (*c*) the ability to reproduce and the subsequent rapid growth of the young was observed on non-treated rabbits.

The question of the variation of sleeping time following the repetition of a uniform dose of Nembutal was first investigated. Fitch and Tatum (3) among others, have shown that the M. L. D. (*i. e.*, dose which kills 50 per cent of the animals) for

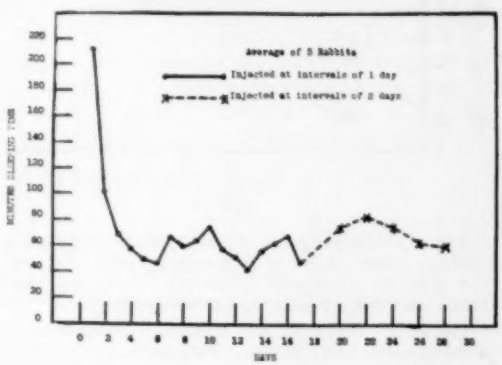

Fig. 1.—Showing the average sleeping times of five rabbits when injected repeatedly with 44 mg. per Kg. of Nembutal dissolved in 0.9 per cent NaCl containing 10 per cent ethyl alcohol. Alcohol received was equal to 0.055 Gm. per Kg.

intraperitoneal doses of sodium pentobarbital is equal to 65 mg. per Kg. They further pointed out that a dose of 39 mg. per Kg. was followed by a sleeping period of 2 hours and 48 minutes. Kohn (4) studying the individual susceptibility of rabbits to intravenous injections of Nembutal found that 70 per cent of the M. L. D. usually produced no untoward effect. Accordingly, it was decided to administer a dose of 44 mg. per Kg. daily over a period of several weeks. The results from such an experiment performed on five rabbits appear in Fig. 1. The period during which the righting reflex was lost was used as the criterion of the sleeping time. The individual variation of sleeping times ranged between 165 and 243 minutes the first day and thereafter, between 0 and 186 minutes. As mentioned previously, the zero sleeping times were disregarded in figuring the averages, although including them would only add slightly to the effect observed, *i. e.*, tolerance.

[2] As supplied commercially for veterinary use.

[3] Five-gram calories per minute were used. The results of this study will be published elsewhere.

Following seventeen daily doses of Nembutal a three-day rest period was allowed and then the injection period was increased to two days. Unlike the work reported by Masuda *et al.* (2), where the drug was given intravenously, only a small portion of the tolerance was lost during the three-day interval, as shown by the broken line in Fig. 1. It should be mentioned, however, that they observed less tolerance in the first place, since the average sleeping time after development of tolerance amounted to only about 70 per cent of the original. The explanation for this apparent discrepancy will be given later in this paper.

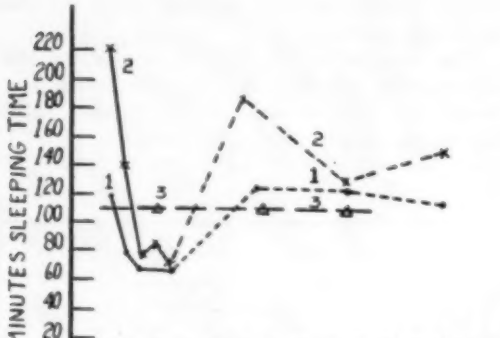

Fig. 2.—Showing the average sleeping times of rabbits when injected repeatedly with 44 mg. per Kg. of Nembutal dissolved in 0.9 per cent NaCl under the following circumstances:

Curve 1. Average of 6 animals. Solid line, no alcohol until the fifth day. Broken line, 0.055 Gm. per Kg. of alcohol daily until the seventeenth day with Nembutal on the eleventh and seventeenth days only. No injections until the twenty-fourth day at which time alcohol and Nembutal were given.

Curve 2. Average of 3 animals. Solid line, +0.055 Gm. of ethyl alcohol for four days. No alcohol on the fifth day. Broken line, same as the broken line in curve 1.

Curve 3. Average of 5 animals. 0.055 Gm. of ethyl alcohol daily for three days with the Nembutal made up in alcoholic saline on the fourth, eleventh and last days only.

Although Dille and Ahlquist (5), who studied the synergism between intravenous injections of ethyl alcohol and Nembutal in rabbits, found that doses of alcohol as low as 0.055 Gm. per Kg. of body weight (the amount used throughout this study) did not increase the sleeping time of these animals appreciably, it was thought advisable to check these results.

One group of rabbits (I) composed of six animals was given a dose of Nembutal made up in 0.9 per cent NaCl while a second group of three animals (II) was given a similar injection of Nembutal dissolved in 0.9 per cent NaCl containing 10 per cent ethyl alcohol. The average sleeping times of these two groups of rabbits were 117 and 220 minutes with an extreme range of 98–138 minutes and 201–245 minutes, respectively (Curves 1 and 2, Fig. 2). The average sleeping time of group II corresponds quite closely to the larger group of similarly treated animals reported upon above. (Fig. 1.) In view of the rather marked synergism observed, a few further experiments were conducted to evaluate the effect of the alcohol on repeated anesthesia.

The same doses were continued for four days and on the fifth day the group (I) which had received no alcohol was given Nembutal made up in 10 per cent alcoholic saline and the group (II) which had received Nembutal and alcohol was injected with Nembutal solution containing no alcohol; in other words, the solutions were given in reverse order. It will be seen from Fig. 2 that not only was the sleeping time almost identical by the third and fourth day, but when alcohol was included in the injection for the first time (the fifth day) for group I, it then did not potentiate the action of the Nembutal at all. Furthermore when 0.055 Gm. of alcohol per Kg. was given to both groups of rabbits at daily intervals until the tenth day and then Nembutal in 10 per cent alcholic saline solution was given, the average sleeping times increased only to about the original time observed for the Nembutal without alcohol. This was confirmed by repeating the alcohol only, until the seventeenth day and again giving Nembutal and alcohol. After this the average sleeping times of both groups following abstinence of any injections for a week was determined and found to be 109 minutes.

Thus, not only does alcohol alone prevent the return of any potentiation but no sign of potentiation is observed following complete freedom from injections for a week.

Since it was shown that Nembutal alone would overcome the synergism, one further experiment was performed to see whether previous injections of alcohol alone would reduce the sleeping time when Nembutal was given in alcoholic saline. A group (III) of five rabbits was injected daily for three days with 0.055 Gm. of alcohol per Kg. and on the fourth day they were given 44 mg. per Kg. of Nembutal as well as the alcohol. Their average sleeping time was 110 minutes. After two seven-day intervals during which they were not treated they again received Nembutal and alcohol and the average sleeping time was 107 minutes both times (Curve 3 of Fig. 2).

From the above results it would seem that there is a marked initial potentiation between alcohol and Nembutal, even for the relatively low doses of alcohol studied, and, moreover, that this synergism disappears after the first several repetitions of the two drugs given either together or separately. However, from the standpoint of repeated anesthesia the alcohol would not appear to alter the results as measured by sleeping time.

One reason for the fact that Dille and Ahlquist report less potentiation from the 0.055 Gm. of alcohol per Kg. of body weight than was observed

in the present investigation may be that—as they state—"Habituation was avoided by allowing at least two days and usually longer between each injection." Since the experiments reported above show that even a week's rest period is not sufficient to allow the reappearance of potentiation, accurate results—at least at low alcohol levels—can probably be obtained only with fresh animals.

The reason for the apparent discrepancy between the work of Masuda *et al.* and that reported in Fig. 1 now becomes evident, namely, the alcohol greatly increased the initial sleeping time. If instead of using the data from Fig. 1 for comparison with the data presented by the above workers, the non-alcoholic injection times of Fig. 2 are used, the results then agree satisfactorily.

With the foregoing information available the second problem, *viz.*, the amount of anesthetic needed to produce a particular sleeping time could be considered. Thirty-seven rabbits were divided into three groups *A*, *B* and *C* containing nine, sixteen and twelve animals, respectively. The injections were carried out in such a way that each animal received an anesthetic every other day for a two-week period and rested one week, after which the cycle was repeated. Treatment days were alternated, so that one group of animals only was injected every day. The one week of rest out of every three probably lessened the number of animals dying as a result of too frequent anesthesia and, no doubt too, decreased somewhat the amount of anesthetic required.

It was desired to anesthetize the animals in group *A* for one hour and those in groups *B* and *C* for two hours. Obviously, the sleeping time for any one animal would vary considerably around these exact periods. After gaining some experience, especially in adjusting the quantity for additional injections in groups *B* and *C*, it became possible to obtain fairly consistent average sleeping times.

From Fig. 1 it was seen that the duration of anesthesia in rabbits from Nembutal injections dropped off quickly following the first two repetitions of the drug, and that once tolerance had developed, and the frequency of injection had been reduced to one dose very other day, the average sleeping time was about 70 minutes. It was further learned that the length of sleeping time could not then be materially augmented by unrestricted increase of the dose without greatly endangering the life of the animal. In other words, after several injections of Nembutal, the sleeping time decreased much faster than the resistance of the animal to larger and larger initial doses increased. Accordingly, it was necessary to administer two or more separate injections to increase the sleeping time to the two-hour period. Unlike the other barbiturates used, Nembutal did not produce any signs of prolonged depression after the rabbits first aroused from total anesthesia. In fact complete recovery as measured by ability to hop, eat, etc., appeared to take place almost as rapidly after two or even three doses as after one injection.

The first injection of from 40 to 50 mg. per Kg. of sodium pentobarbital was given each day while second and third doses varied in size from about ten to twenty-five mg. per Kg. of body weight, depending upon the previous record of the particular animal. An exception was made to these dosages in the case of the first several days of treatment because the animals would then sleep considerably longer than the desired periods. In these cases slightly less anesthetic was employed. Caution governed all of the decisions regarding dosage as the continued life of the animal then was the main objective.

The reactions of different animals varied considerably and even those of any one animal seemed to change from week to week. Several examples fairly typical of the dose of Nembutal used and the resulting sleeping time for the two periods studied are given in Table I. This table illustrates the need for two and sometimes three injections in the case of the two-hour period animals. The second injection was given after 5 or 10 minutes in the cases where the animal would not go to sleep, as for example the ninth and eleventh day for animal *Ch*.

Altogether over 3000 injections were given the

Table I.—Showing Examples of the Sleeping Time for One Complete Cycle (Not the First) in Response to Repeated Injections of Sodium Pentobarbital in Animals being Treated Every Other Day for Two Weeks Following a Week's Rest Period

One-Hour Period			Two-Hour Period		
Day (Animal *GC*)	Dose, mg./Kg.	Sleeping Time, Minutes	Day (Animal *W*)	Dose, mg./Kg.	Sleeping Time, Minutes
1	43	75	1	41	118
3	43	55	3	41 + 11	77 + 45 = 122
7	43	70	7	41	105
9	44	57	9	41 + 22	59 + 81 = 140
11	44 + 19	15 + 58 = 73	11	41 + 22	65 + 55 = 120
		Average 66			Average 121
(Animal *Ch*)			(Animal *Q*)		
1	41	58	1	45 + 27	72 + 53 = 125
3	41	65	3	47 + 27	65 + 71 = 136
7	41	98	7	47 + 22	85 + 40 = 125
9	41 + 12	0 + 55 = 55	9	47 + 24 + 16	70 + 27 + 35 = 132
11	41 + 15	0 + 47 = 47	11	50 + 8 + 27 + 19	0 + 30 + 55 + 40 = 125
		Average 65			Average 131

Table II.—Showing the Amount of Sodium Pentobarbital Required to Produce Repeated Anesthesia for Two Periods: Group *A*—75 minutes—and Groups *B* and *C*—Approximately 2 Hours

Group	Number of Anesthesias	Average Dose, mg./Kg.	Average Sleeping Time, Minutes	Sleeping Time per mg./Kg., Minutes	Number of Animals, Orig.-Final	Period of Treatment, Days
A	319	44.6	74.5	1.66	9–4	242
B	553	63	122	1.93	16–5	221
C	306	68	123	1.80	12–3	201

three groups of rabbits throughout a period of almost a year. With the exception of the first several weeks, during which period the maximum sleeping time was not recorded, the observations from these anesthesias have been assembled and are summarized below.

The distribution of the dosage having been considered, there will now be discussed the amount of anesthetic required on the average to produce sleep for (*a*) slightly over an hour, and (*b*) a two-hour period. The average dose of the barbiturate and the average sleeping times for all of the anesthesias have been determined, and from these the sleeping time per mg. per Kg. of body weight has been calculated. These data are set forth in Table II.

It will be seen from this table (column 5) that relatively more anesthetic is required to produce sleep for the shorter period. This was to be expected, and merely is evidence that a threshold dose must be given before sleep is produced at all. Thus, when the animal awakens, about half the original dose will produce anesthesia for a time approximately equal to the first period.

It is obvious that the averages appearing in Table II are only valid when there has not been a material or progressive change in susceptibility. To check this point the average daily sleeping time per unit dose of anesthetic was determined and plotted against the day the anesthetic was given. (Fig. 3.) An arbitrary dose of 44 mg. per Kg. was selected for the unit dose for illustrative purposes as this was the dose used previously for the determination of tolerance. (Fig. 1.) Thus the sleeping times of rabbits in group *A*, which actually received 44.6

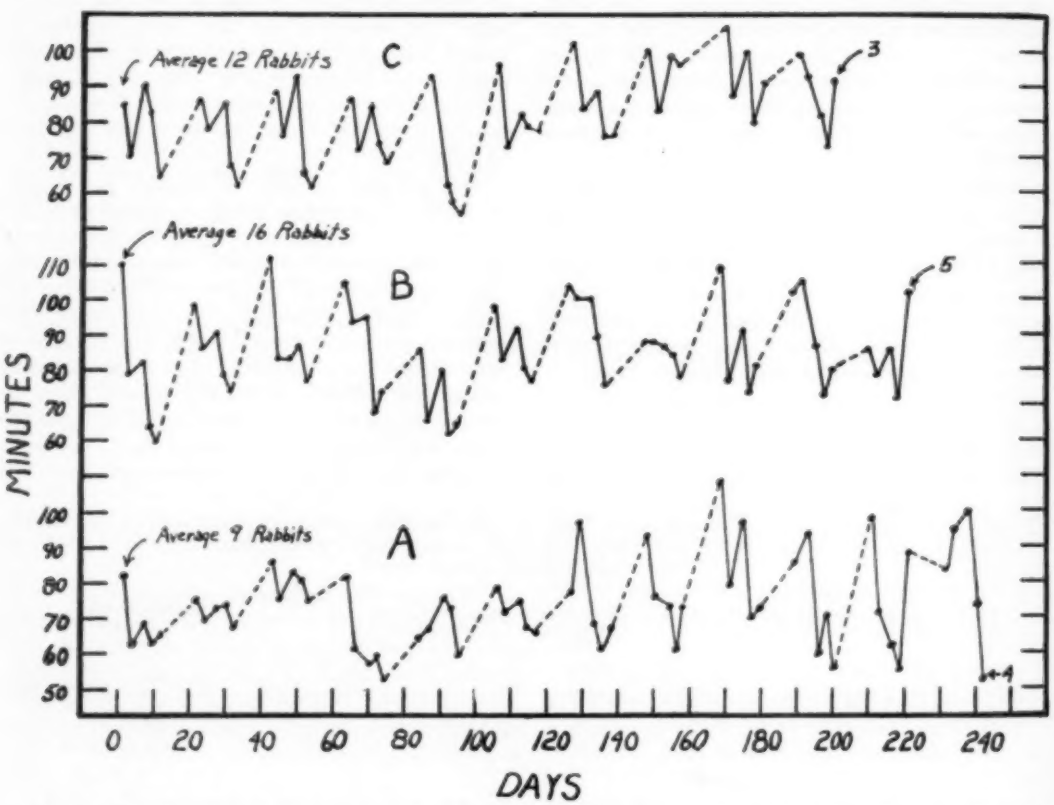

Fig. 3.—Showing the relative average sleeping times of three groups of rabbits injected repeatedly with Nembutal.

The dose given each group was different (see Table II), but the sleeping times have been adjusted for a standard dose of 44 mg. per Kg. The anesthetic was given on Tuesday and Thursday of the first week, and Monday, Wednesday and Friday of the second week, then a week's rest period (dotted line) was allowed.

mg. per Kg., are the only ones which required little mathematical adjustment, and hence, they represent the only case where the comparative sleeping time is practically identical with the actual sleeping time. Groups *B* and *C*, on the other hand, received on the average 63 and 68 mg. per Kg. as reported in Table II and thus, for comparative purposes, required a proportionate correction to the lower dosage of 44 mg. per Kg. As reported above, the sleeping time does not strictly follow the dosage. Therefore the corrected values are comparative only and tend to be high. In any case, however, the values shown represent correctly the condition of the rabbits in regard to susceptibility; and moreover the absence of a continued slope for each group of animals indicates that there has been no change in this characteristic. The lack of increased tolerance after long periods of administration demonstrate an absence of habituation to the drug—which is of interest from a clinical point of view.

Table III.—Comparing the Lengths of Sleeping Time of Male and Female Rabbits when Injected Intraperitoneally with Sodium Pentobarbital. All of the Rabbits Have Been Subjected to Repeated Anesthesia

Sex	Date	Number of Rabbits	Average Dose, mg./Kg.	Average Sleeping Time, Minutes	Average Sleeping Time per mg./Kg., Minutes	[a]Mean Average Sleeping Time per mg./Kg., Minutes
Males	March 29	19	70.0	118	1.68	1.59
	March 31	19	76.0	114	1.50	
Females	March 29	13	64.5	105	1.63	1.54
	March 31	13	71.0	103	1.45	

[a] The dates used in this table represent the fourth and fifth treatment days of the three week cycle.

In general, the variation in sleeping time for any one period appears to parallel the frequency of the anesthesias. Thus the general trend seems to be from a rather long sleeping time to a somewhat lesser one as a result of the Tuesday and Thursday doses. Then a longer sleeping time is seen as a result of the four-day interval until Monday, with a continued lessening of the duration of anesthesia following the Wednesday and Friday injections. Finally, a return to a fairly constant level occurs with the week's rest period. This cyclic behavior appears to be more evident in groups *B* and *C*.

Another important factor is the relative response of male and female animals to repeated anesthesia. Neither Kohn nor Fitch and Tatum were able to demonstrate any difference in the sleeping periods between the two sexes following a single injection of Nembutal in rabbits. That their finding is confirmed for repeated anesthesia is apparent from the results appearing in Table III. Here the average sleeping times of male and female rabbits are seen to correspond to within 3 per cent. There were equal numbers of one-hour period animals of each sex included in this tabulation. Still further confirmation was found in a smaller series of animals, where the average sleeping times following the first injection of Nembutal (44 mg. per Kg.) were 197 and 193 minutes for male and female rabbits, respectively.

Finally, attention is called again to the significant question of survival. It will be remembered that the prime objective of this study was to find an anesthetic which would be fairly innocuous when treating animals over a period of approximately one year. How well this objective was accomplished may be seen from Fig. 4 which shows the mortality of the rabbits in groups *A*, *B* and *C*. Regarding the cause of death the following breakdown will be of interest. Two of the five animals which died from causes not associated with the anesthetic were killed after 48 weeks of repeated anesthesia; one died from a testicular infection; and two died from unknown causes. Of the remaining thirty-two rabbits, twelve were still living after 50 weeks of treatment, while twenty died of the usual respiratory collapse without regaining consciousness, shortly after receiving an injection of Nembutal. Deaths, rather oddly, occurred with about equal frequency on each of the five treatment days, and would sometimes follow a dose of the barbiturate which two days previously would scarcely put the same animal to sleep. On the other hand, a rabbit would occasionally become extremely refractory, and after giving it three injections without producing sleep, the animal would be returned to its cage for that day. Kohn has made similar observations on the change, from time to time, in susceptibility of individual rabbits. Only once did a rabbit succumb to any injection following the initial daily dose of Nembutal,

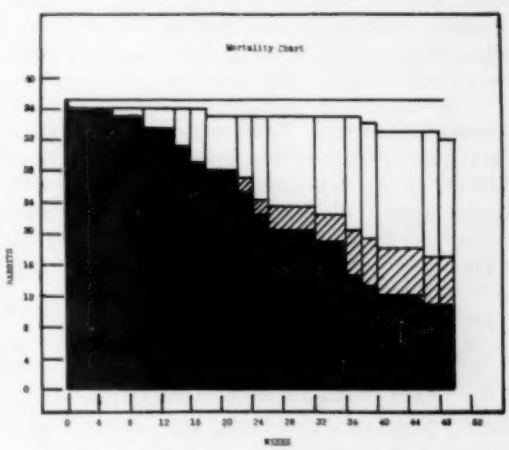

Fig. 4.—Showing the mortality of the rabbits given long repeated anesthesias.

Solid black. Animals living.

Cross hatch. Animals dying at the time of anesthesia but suffering from "snuffles."

Open block. Animals dying at time of anesthesia.

Open. Animals killed deliberately or dying from causes not associated with the anesthetic.

and no death was associated with loss in weight. It may be that decreasing the amount of hay in the rations increased the mortality.

On several occasions, animals were saved from death by artificial respiration, in some cases supplemented with coramine. Better results could probably have been obtained with the use of the other analeptics, metrazole or picrotoxin. However, with a limited personnel and with four animals being used simultaneously, the close observation required to see every case of failing respiration in time for prophylactic measures was impossible.

While it was hardly to be expected that the periodic examinations made of various parts of the eye would reveal any pathology, there was a possibility of damage to other bodily organs as a result of the frequent repetition of the anesthetic. Dr. Kohn-Richards kindly made a histological study of several of the tissues from three of the rabbits which had received injections of Nembutal; his report is as follows:

"Rabbit *V*, injected from February 20 to November 17—killed November 18. Received 8.6 Gm. of sodium pentobarbital total. Liver, cloudy swelling, no fat. Kidney, severe cloudy swelling, hyperemia, no fat. Heart, cloudy swelling.

"Rabbit *Ch*, injected from December 31, 1938 to November 16, 1939—killed November 18. Received 8.3 Gm. of sodium pentobarbital total. Kidney, no fat, cloudy swelling. Liver, moderate fat infiltration, possibly a normal limit, marked cloudy swelling, some round cell infiltration around the bile ducts which may not be significant.

"Rabbit *B*, injected from January 12 to November 29—killed December 8. Suffered from 'snuffles' for from 4 to 6 weeks before being killed. Liver, fat moderately present, hyperemia, distension of sinusoids, marked cloudy swelling. Heart, cloudy swelling. Kidney, no fat, tubuli epithelium markedly swollen and indistinct, severe cloudy swelling, some places showing early necrosis.

"The last animal undoubtedly shows the most pronounced changes. However, since according to the protocols, the animal suffered from snuffles, it is doubtful if the changes can be attributed to the drug or solely to the drug. The changes in the other two animals are of a nature that one might expect the organs to return to the normal status and do not seem to indicate irreparable damages."

CONCLUSIONS

1. As a result of several daily intraperitoneal injections of 44 mg. per Kg. of Nembutal, dissolved in 0.7 ml. of 0.9 per cent NaCl containing 10 per cent ethyl alcohol, the sleeping time of rabbits decreases abruptly from approximately $3^1/_2$ to $1^1/_4$ hours. When the alcohol is omitted from the solution the first one or two sleeping times only are shortened.

2. The use of Nembutal intraperitoneally for long-repeated anesthesia in rabbits is practical if some degree of care is used in adjusting the dosage to the requirements of the individual animal.

3. There is no evidence of habituation of rabbits to Nembutal even when the drug is given repeatedly over a period of one year.

4. To obtain anesthesias in rabbits of one to two hours' duration, with minimal risk, it is necessary to give repeated small doses of the drug.

5. An average initial dose of 40–44 mg. per Kg. intraperitoneally is satisfactory for this type of anesthesia in rabbits.

6. The sex of the rabbits does not alter the sleeping time for either single or repeated injections of Nembutal.

7. The liver, heart and kidney appear to suffer mild but not irreparable injury as a result of long-repeated anesthesia.

The author wishes to express his gratitude to Miss Barbara Petty for her assistance throughout this work and to Dr. Charles F. Kutscher who made the study possible through his liberal support of this laboratory.

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NOTICE

There has been some adverse criticism of some of the illustrations which have appeared in the JOURNAL recently. Authors can assist in allaying this criticism by adhering strictly to the directions on the preparation of papers given under "Notice to Authors."

Potentilla Anserina in Essential Dysmenorrhea

By A. Richard Bliss, Jr., and Collaborators†*

During some collaborative studies of the Viburnums, interest was aroused in *Potentilla anserina* as an agent of possible use in the treatment of essential dysmenorrhea. To insure against errors in identity, it was decided to secure a supply of the crude drug, and to manufacture an extract from it for use in the investigations herein reported. Great difficulty was experienced in securing authentic *Potentilla anserina*. A number of samples were submitted by several crude drug houses, and these were turned over to Dr. Heber W. Youngken, pharmacognosist of the Massachusetts College of Pharmacy, for identification. Dr. Youngken found that each of the first samples submitted was spurious. Most of them were *Potentilla argentea*, and Dr. Youngken suggested a possible explanation for this confusion (1). The old American Code name for *Potentilla anserina* is *Argentina Anserina* (L.) Rydb., and this fact possibly explains why *Potentilla argentea* has been collected by some who thought the two plants were identical. The American Code of naming plants followed by Rydberg, Small, Britton and Brown, and others was ruled out at the last meeting of the International Botanical Congress, the type species being alone continued.

Although *Potentilla anserina* is listed as occurring from New Jersey northward and westward (2), it appeared impossible to secure an unadulterated supply from domestic sources. (During the week of July 9, 1939, Dr. Youngken (5) reported that he saw *Potentilla anserina* in fair amounts growing in gravelly soil along the Aroostook and Rustic Rivers in Aroostook County, Maine.) After 14 months, S. B. Penick and Company was finally successful in securing an authentic lot from a foreign source. The identity of this lot was confirmed by Dr. Youngken.

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The literature has disclosed very few references on the action of *Potentilla anserina*. Maisch (3), in a paper entitled "On the Constituents and Properties of the Genus *Potentilla*," states: "*Potentilla anserina* L. (Silver Weed). Both the herb and perennial root have a mild astringent taste, and are said to have been used by the Indians as an antidote to snake poison; while in Europe it was employed in diarrhea, hemorrhages, pulmonary complaints, some hepatic disorders and dropsy." Le Maout and Decaisne (4) simply state: "Roots and Leaves astringent."

On the basis of animal experiments in which muscular cramps artificially produced by barium were relieved by *Potentilla anserina* (7), Hauptstein (6) tried the drug clinically in dysmenorrhea. Capsules containing 0.25 and 0.5 Gm. were given three times a day, one, two or three capsules at a time, from two to three days before menstruation through the first part of the period. Pain was prevented in several cases, but generally only for the period during which the drug was administered. In a few cases, however, dysmenorrhea seemed permanently cured by treatment with the drug before and during a single period. Failure was reported only in one case of a hypoplastic uterus, one myoma case and 2 cases where the anatomical findings were negative. Hauptstein recommended the drug because of its cheapness and low toxicity.

For the sake of clarity, Beckman's (8) practical statements concerning essential dysmenorrhea are introduced here. "*Essential Dysmenorrhea* (Painful Menstruation): Perhaps more than 50 per cent of women suffer from dysmenorrhea, the symptoms varying from a mild physical and mental discomfort that is little greater than the normal depression during the period, to severe attacks of pain that rack the patient's back, head, legs and lower abdomen for several days and leave her in a state of great fatigue during the early part of the intermenstrual period. Grant that dysmenorrhea is only a symptom and not a disease, and classify the various types on an alleged causative basis, the fact will remain that there is a constitutional something under-

lying all the cases; that is to say, that the dysmenorrheic woman differs in some essential way from the nondysmenorrheic. This position is of course challenged, and the attempt is made to find a neuralgic, ovarian, congestive, obstructive, inflammatory, mechanical, autonomic, endocrine or allergic cause in all cases—which would be very fine were it not for the fact that the very same *causes* are found in many individuals who do not have dysmenorrhea. Therefore, in the present state of our knowledge, the term 'essential dysmenorrhea' would seem to be permissible."

EXPERIMENTAL

Material Used.—An extract, representing four times the concentration of the dried crude drug, was manufactured from the dried authentic *Potentilla anserina*. Compressed tablets, each containing three grains of the extract, were manufactured from this extract of *Potentilla anserina*. No other vegetable, animal or chemical physiologically active drugs were added to the extract or to the tablets, and the tablets were not coated. Each of these compressed tablets of Extract of *Potentilla anserina* (3 grains) represented 12 grains of the powdered drug.

Toxicity Experiments on Animals.—Doses of from 4 to 8 tablets were administered to adult albino rats, guinea pigs and rabbits over a period of 10 to 14 days. These doses represented from 48 grains to 96 grains of the powdered crude drug, and from 12 grains to 24 grains of the Extract of *Potentilla anserina;* the maximum dose administered to human subjects by Hauptstein (6) being 24 grains of the powdered crude drug 3 times a day.

There was no evidence of any harmful effects on any of the experimental animals, and autopsies failed to disclose any deleterious effects on any of the internal structures of the animals. In fact, the animals would eat the tablets voluntarily, and did not appear to develop any dislike for food with which crushed tablets had been mixed.

The work of Schneider and Nevinny (7) was confirmed by these authors, using guinea pigs as the experimental animals.

Investigations on Human Subjects.—Two staff members took two tablets three times a day for four days without any evidence of ill effects, before the clinical studies on patients were started.

Eight physicians coöperated in making a clinical study of the Compressed Tablets of Extract of *Potentilla anserina*. These gentlemen were provided with a supply of the Compressed Tablets, and were sent the information set forth above.

Each physician's attention was called also to the following: "This drug is commonly known as 'Silver Weed.' You will note that Hauptstein (6) used from 4 to 8 grains in each capsule, and that the dose was given 3 times a day, and varied from 1 to 3 capsules. It appeared to us that this was rather bulky, and therefore we decided on an extract of the drug which represents 4 times the activity of the crude drug; thus, decreasing the actual bulk by about one-fourth. In short, the dose of the extract is then $1^1/_2$ grains (representing 6 grains of the drug) to 6 grains (representing 24 grains of the drug), 3 times a day. The tablets of the extract which have been made up are 3-grain tablets, and consequently, the *average dose* of the extract would be *from $^1/_2$ to 1 tablet*, 3 times a day. The *maximum dose* as given by Hauptstein (6) was 24 grains of the powdered drug 3 times a day (3 capsules each containing 8 grains of the powdered drug). Two of the tablets of the extract represent 24 grains of the powdered drug.

"It is interesting to note, in view of the prolonged delay (something over a year) in getting any material from Germany (or elsewhere), that one of the German firms (9) has just recently come out with a preparation of this drug. This manufacturer claims that clinical tests are in agreement with the results of animal experimentation; that the drug is safe and sure with all spastic dysmenorrheal conditions; that with the majority of dysmenorrheal cases a quick and positive effect is obtained; that there are no side effects; and that even in large doses the drug may be used without hesitation. They advise using the drug several days before the period, but that even if the period has started the drug will give prompt relief from pain."

Clinical Results Observed.—After a period of several months had elapsed, each collaborating physician was provided with an adequate supply of forms similar to the copy which follows, with the request to return the same as soon as possible.

Clinical Data on *Potentilla Anserina*

1. Patients name, initials or number:_____
2. Age:____ 3. Social state: (*a*) S____ (*b*) M____ (*c*) W____
4. Has patient ever been pregnant____ Number of pregnancies____
5. Age at onset of menses____ 6. Frequency of periods____
7. Are periods regular____ irregular____
8. Number of days of average flow____
9. Severity of pain: (*a*) mild and continues work____ (*b*) moderately severe but continues work____ (*c*) forced to quit work or to go to bed____
10. On what day of period is pain most severe____
11. Results obtained with *Potentilla anserina:*
    (*a*) complete relief_____
    (*b*) partial relief_____
    (*c*) no relief_____
12. Were there any untoward or disagreeable side effects from the drug____ If yes, describe, briefly_____

13. Dosage and frequency of administration_____

14. Total number of tablets given_____
15. Physician's name or initials_____

From the foregoing data, the following summary of the same is of interest:

Number of patients studied: 25.
Single: 15; Married 8; Widows: 2.
Number who had been pregnant: 3.
Number with irregular periods: 8.
Number with regular periods: 17.
Number having mild pain and continue work: 2.
Number having severe pain but continue work: 7.
Number forced to quit work or go to bed: 16.
Number showing complete relief after *Potentilla anserina:* 8.
Number showing partial relief: 14.
Number showing no relief: 3.
Number showing side effects: 2 with dryness of mouth; 1 sleepy; 2 nausea, but probably not due to drug.
Dosage: 1/2 tablet: 3; 1 tablet: 17; 2 tablets: 5.
Total number of tablets taken: 1 1/2: 1; 6: 2; 9: 1; 12: 15; more than 20: 6.

The three social states were represented; also patients who had been pregnant. Sixty-eight per cent of the patients were regular, and 32% were irregular. Four per cent had mild pain but continued work; 32% had severe pain but continued work; and 64% were forced to quit work or to go to bed. Of these patients 32% were completely (one "almost completely") relieved of discomfort and pain; 56% were partially relieved; and 12% showed no relief (one of these three patients later showed pathology of the cervix).

From what is known of the action of *Potentilla anserina*, it is probable that the dryness of the mouth mentioned by two patients, the sleepy feeling mentioned by one patient and the nausea referred to by two patients were not due to *Potentilla anserina*, but were probably psychic.

It is to be noted that the single dose ranged from 1/2 tablet to 2 tablets, and it is possible that some of the patients who were only partially relieved of discomfort and pain might have been completely relieved with larger dosage; this might be true also of 2 of the 3 unrelieved patients. It is regrettable that a larger supply of tablets were not available so that this point might have been definitely established.

Patients took from a total of 1 1/2 tablets to as many as 40 tablets. This gave opportunity for observance of patients who had been on *Potentilla anserina* for longer periods. As yet, the time of experimentation has prohibited adequate observations which would warrant any statement as to the possibility of permanent cures. It is obvious, however, that in 88% of the patients either complete (32%) or partial (56%) relief was obtained. From the data presented it is obvious, too, that *Potentilla anserina* is of material use for relieving the pain and discomfort which may attend the condition under discussion, without the aid of any other drug agents. The obvious advantages of this fact need no comment, although combinations with other drugs might be resorted to in certain cases. Although the work completed has not established the probability of permanent relief, the advantages of having a non-narcotic, non-coal-tar agent for such application are great indeed. Many of the physicians and still more of the patients are desirous of securing additional supplies of the agent. Calls for the tablets continue to come in from the collaborating physicians, as well as from physicians who have not used the drug but have heard of it during casual conversations with the men who have experimented with the tablets. Some of them have said that even in the event *Potentilla anserina* is not a permanent cure, nevertheless the relief afforded even temporarily (during menstruation periods) warrants its existence and use, for some of these patients who have been relieved by *Potentilla anserina* heretofore got relief only from a hypodermic injection of one of the narcotic drugs.

Further clinical investigations are being carried out in two of the larger medical centers of the country. An investigation of the constituents of *Potentilla anserina* is under way. The authors wish to express their thanks to Dr. Heber W. Youngken for his valuable assistance.

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NOTICE

Delays in publication are frequently due to the fact that authors fail to carefully check the list of references. The editor cannot be expected to supply missing parts of references.

Female Hormone in Bituminous Coal from Shangtung Province

*By T. H. Tang, W. C. Wang and C. C. Peng**

The study of female hormones becomes more and more intensive, as its clinical applications in the treatment of diseases of women become more and more important. The sources of the female hormone are urine of pregnant and non-pregnant women, urine of pregnant mares, corpus luteum, placenta, etc. But several workers have reported that extracts of various plant products contain the active substance. Walker and Janney reported positive results with extracts of elder leaves and catkins, willow catkins, sprouted oats and rhuba leaves. In a recent publication, Butenandt isolated an active substance from palm leaves. Plants are considered to be the origin of coal, and the latter might, therefore, still contain some active substance from the original plant.

EXPERIMENTAL

The authors analysed eight samples of bituminous coal from different localities in Shangtung province and tested the active substance from each sample. The results are reported below:

Preparation of the Active Extract.—One Kg. of powdered coal was digested with sulfuric acid on a water-bath for six hours. After filtration, the filtrate was extracted with ether. The ether solution after washing with 10% sodium carbonate solution and drying with anhydrous sodium sulfate, was evaporated *in vacuo* and the residue was taken up in 20 cc. of olive oil.

Determination of Potency.—Allen and Doisy (1) have called the amount of potent substance necessary to produce a full change from the negative to the estrual vaginal spread in a castrated rat of approximately 140 Gm. (±20 Gm.) in weight a "rat unit" (R. U.). The above prepared olive oil solution was divided into several doses, which were injected into the castrated rats. After 48 hours the vaginal smears of the test animals were examined under the microscope to ascertain the estrual condition. The results are tabulated below:

Sample	R. U./Kg.
(1) Huang-Shan	100
(2) Li-Tsia-Ku	80
(3) He-Shan	200
(4) Hsin-Tsing	60
(5) Huang-Tsia-Ta-Wa	50
(6) Ta-Wa-Tsing	40
(7) Tao-Hau-Ku	65
(8) Tsao-Yang-Po	45

SUMMARY

1. The potency of the eight samples from Shantung Province have been determined.

2. Sample (3) He-Shan posessed the highest potency (200 R. U./Kg.) while sample (6) Ta-Wa-Tsing possessed the lowest (40 R. U./Kg.)

Table I.—Proximate Analysis of Samples of Shangtung Coal

Sample	Moisture, %	Ash, %	Volatile Matter, %	Fixed Carbon, %	Calorific Value (Cal.)
(1) Huang Shan	0.49	9.48	7.93	80.30	8260
(2) Li-Tsia-Mu	0.25	7.78	8.56	83.40	8460
(3) He-Shan	0.41	7.52	16.83	75.24	8060
(4) Hsin-Tsing	0.51	10.24	13.89	75.36	7960
(5) Huang-Tsia-Ta-Wa	0.35	9.35	16.01	74.30	7600
(6) Ta-Wa-Tsing	0.52	13.05	16.49	69.95	7464
(7) Tao-Hua-Ku	0.62	16.21	14.49	68.86	7232
(8) Tsao-Yang-Po	0.74	19.97	15.59	63.82	6410

Table II.—Elementary Analyses of Samples of Shangtung Coal

Sample	C %	H %	O %	N %	S %
(1) Huang-Shan	80.63	1.90	17.47	0.46	2.48
(2) Li-Tsia-Ku	83.24	1.89	14.87	1.24	3.21
(3) He-Shan	80.83	2.09	17.08	1.28	0.64
(4) Hsin-Tsing	78.60	2.05	19.36	1.25	3.69
(5) Huang-Tsia-Ta-Wa	78.93	2.14	18.93	1.38	2.11
(6) Ta-Wa-Tsing	76.00	2.23	21.77	1.35	0.79
(7) Tao-Hua-Ku	73.69	1.82	24.40	1.27	0.75
(8) Tsao-Yang-Po	68.72	2.06	29.22	1.25	0.83

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Pectin Studies, V. Organic Base Derivatives of Pectinic and Pectic Acids

By Reinhold F. Stuewer and Aksel G. Olsen**

The present conception of pectin as a chain molecule made up largely, if not entirely, of galacturonic acid anhydride units, part of which are methylated, greatly simplifies an interpretation of pectin properties (2), (6), (8), (9). Acid properties are conferred by the free carboxyl groups resulting from partial demethoxylation. The reactions of pure pectin correctly called pectinic acid are, therefore, essentially those of a typical organic acid of high molecular weight (1). The colloidal properties presumably are indicative of the number of units in the chain, and the only difference between pectic acid and pectinic acid is that the former is more completely demethoxylated than the latter. Both pectins and pectic acid of greatly varying molecular size, as indicated by specific viscosity and gel properties, do exist; but usually pectic acid will be found to be of considerably lower molecular weight as indicated by specific viscosity, than is normal for pectin. This is a normal result of conditions which cause demethoxylation. However, by slow, strong acid, low temperature demethoxylation one can avoid the splitting of the long chains (7). In this manner pectic acid of high molecular weight can be prepared, while by the usual alkaline method of demethoxylation low molecular weight pectic acid usually is obtained.

The reaction of pectin and pectic acid with certain metal ions to form insoluble compounds has long been known. Such insoluble precipitates have been described by a number of writers from Braconnot to von Fellenberg. From a study of several pectins differing in methoxyl content, von Fellenberg correctly concluded that "the more methoxyl groups split off the more acid groups present in the pectin molecule and the easier does coagulation with metal salts occur" (5).

Nevertheless, even more recently, some have failed to recognize the essentially stoichiometric relationships which control reactions that occur between pectin and either inorganic or organic bases. Some commercial pectin preparations may be but slightly acid in reaction due to a neutralization of the free acid groups. However, the combined cations are readily removed by washing with acidified alcohol and the excess acid can be removed by washing with dilute alcohol. Such pectin in 1% solution will have a p_H well below 3.0, and should preferably be referred to as pectinic acid. It is questionable whether a "neutral" pectin, that is a completely methylated pectin, exists in the free state. Most pectin samples observed by the authors have equivalent weights of not over 1200, and some commercial preparations run as low as 400 equivalent weight while pectic acid has an equivalent weight of about 205. It is not logical to assume a difference in type of reactions entered into by pectic acid and such pectinic acids. The difference is rather one of degree due to the difference in equivalent weight. Naturally one may expect pectic acid to form insoluble precipitates with many salts which do not precipitate pectins of high equivalent weight because the latter do not combine with enough of the precipitating ion to become insoluble. For example, pectin of high combining weight reacts with calcium to form calcium pectinate although it does not form a precipitate with calcium, whereas insoluble calcium pectate provides the well-known method for determining pectic acid.

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Nevertheless, Chodnew (4) many years ago prepared a pectinic acid, which he assumed to be pure pectin, and which was precipitable with calcium. In corroboration of this, the present authors have observed that pectins of about 475 equivalent weight or less are readily precipitable by calcium. Other metallic ions may form precipitates with pectins of higher equivalent weight, for example aluminum, copper, iron, nickel and others. This, of course, is what might be inferred from von Fellenberg's general statement quoted above. The maximum equivalent weight permitting precipitation differs to some extent, and inasmuch as such precipitates tend to be soluble in acids, the

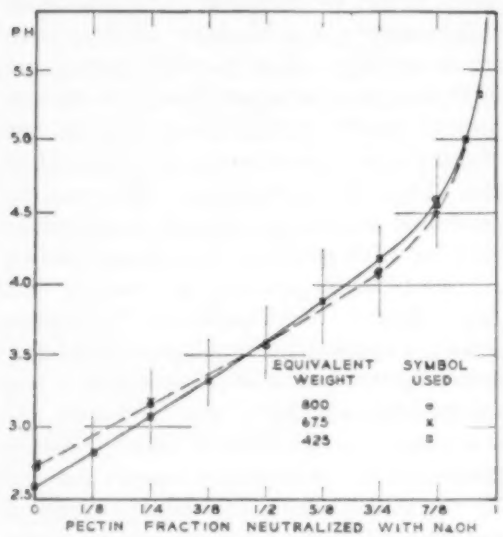

Fig. 1.—Electrometric Titration Curves for 1% Pectin Solution.

maximum equivalent weight at which precipitation occurs will vary with the p_H of the solution, the higher the p_H the easier is the precipitation.

None of these reactions of pectin need to be explained in any but common stoichiometric terms. Under some conditions adsorption may occur in addition to chemical combination (11); but adsorption is not needed to explain and by itself certainly does not explain the reactions of pectin with metallic ions. Pectin is essentially a typical organic acid of high molecular weight. Typical titration curves for one per cent water solutions of pure pectins are shown in Fig. 1.

Some heavy metal ions, as, for example, lead, react so readily with pectin that they can be quantitatively removed from solutions in the pectin precipitate. In fact lead will replace other ions such as calcium so that finely divided calcium pectate preparations if used as a filter bed, or if dispersed in a solution and again filtered off will effectively remove lead ions from the solution. This is readily applicable to other heavy metal ions and it reminds one of Braconnot's early suggestion that pectic acid be taken as an antidote for heavy metal poisoning (3). It also suggests how the ever present pectin in fruits may greatly minimize the danger of lead poisoning from spray residue (10).

The alkali metals and ammonia also react with pectin in stoichiometric proportions but form only soluble salts. These are, therefore, most conveniently obtained by adding calculated amounts of the ions in the form of salts of weak acids to a suspension of the pure pectinic acid, or pectic acid as the case may be, in dilute alcohol. For this purpose the carbonates are most desirable. In 50% alcohol the pectin particles swell sufficiently so that a rather rapid reaction will occur. After the reaction is complete the pectin can be recovered by filtering, then washed with additional alcohol and dried. This method of reacting on powdered pectin while suspended in a solvent in which the pectin particles swell but are not soluble but in which the other reagent is soluble is a very convenient procedure, in particular in the preparation of several organic derivatives of pectin and pectic acid.

The solubilities in water of metal base pectates and pectinates, as already indicated, differ greatly one from another, all the way from completely insoluble to completely soluble. It was, therefore, considered of some interest to observe the differences introduced by forming various organic base derivatives.

EXPERIMENTAL

Although there is nothing particularly novel about neutralizing an organic acid by an organic base we have failed to find any published record of preparations of organic base pectinates and pectates of the general form $R_x(COOCH_3)_n(COOB)_{x-n}$ in which R is the galacturonic acid anhydride unit and B the

organic base. With x the number of galacturonic acid anhydride units in the molecule and n the number of methoxyl groups there would be $x - n$ carboxyl groups free to react with B. In partially neutralized products hydrogen replaces some of the B groups.

We have prepared a number of such compounds and many of the organic base derivatives have been found to have interesting properties. For example, the dispersibility of different pectates in dilute alcohol is greatly modified by the base. The maximum per cent of alcohol in which the common sodium salt of pectic acid can be dispersed to form a clear solution is 6–8%; for the potassium salt, it is 10–12%; for triethanolamine pectate, it is about 60% and for propyl amine pectate, it is about 75%. It is readily apparent that this permits the use of pectic substances under conditions not otherwise possible, as for example in cosmetic, or pharmaceutical preparations containing alcohol in appreciable concentration. Propyl amine pectate also has interesting emulsifying and foam producing properties, and even partial neutralization of pectinic or pectic acids by different organic bases may confer attributes quite different from those of the original material. The now well established value of pectin in the treatment of infected wounds, burns, diarrhea, etc., suggests its utility also in combination with therapeutically important organic bases.

Among the compounds we have prepared are the pectic salts of the following:

Simple aliphatic amines	*Substituted aliphatic amines*
1. *N*-Propylamine	1. Diethanolamine
2. *n*-Butylamine	2. Triethanolamine
3. Di-*n*-butylamine	3. Ephedrine
4. Tri-*n*-butylamine	4. Benzylamine
5. Tri-iso-amylamine	5. Methyl glucamine

Aromatic amines	*Heterocyclic bases*
1. Aniline	1. Pyridine
2. *o*-Anisidine	2. Piperidine
3. *p*-Toluidine	3. Quinine

Guanidines	*Substituted hydrazines*
Diphenyl guanidine	Phenyl hydrazine

In general the dissociation constants of all organic bases suitable for reaction with pectic or pectinic acids fall between the limits of $K = 1 \times 10^{-3}$ and $K = 1 \times 10^{-10}$. Some substituted ammonium hydroxides are even stronger than $K = 1 \times 10^{-3}$ and of course will react with either pectic or pectinic acid.

The reactivity of most of the compounds listed above is readily demonstrated by their ability to disperse pectic acid. That is, while pectic acid is quite insoluble in water nearly all the organic derivatives are very soluble, and the solubilizing of the pectic acid is therefore a definite indication that the reaction has taken place. With some compounds such as quinine and cinchonine, the derivatives although formed are not soluble. The limitations of reactivity may be summarized as follows:

Aliphatic Amines.—The common aliphatic amines are all fairly strong bases. Their dissociation constants fall roughly between 1×10^{-3} and 1×10^{-6}. They react readily with either pectic or pectinic acids to give practically neutral salts.

Some of the higher aliphatic amines, *e. g.*, tributylamine are practically insoluble in water, yet will react with pectic acid to form soluble pectates. Amines of this type which are completely insoluble in water may react readily by use of the dilute alcohol suspension method, since in general they are soluble in alcohol solutions.

Solubility in alcohol or acetone solutions of rather high concentration appears to be a characteristic of all pectic salts of aliphatic amines.

Other Bases.—The strictly aromatic amines, *e. g.*, aniline and toluidine are considerably weaker bases than are those of the simple aliphatic series. Aniline has a dissociation constant of 4.6×10^{-10} but regardless of its low basicity, it reacts to a sufficiently great extent to give a water soluble product with pectic acid, as is readily shown by addition of aniline to a water suspension of pectic acid. Pyridine, which shows all the characteristics of aromatic compounds, behaves in much the same way as aniline, while piperidine, its reduction product, is typically aliphatic. *p*-Phenylene diamine, while of about the basic strength of aniline does not cause appreciable dispersion of pectic acid. Di- and tri-arylamines, *e. g.*, di-phenylamine, are almost without basic properties and do not react. As with other properties benzyl amine behaves as an aliphatic amine. Ephedrine also shows the reactivity one would expect of an aliphatic amine.

Quinine which has a K value of 2.2×10^{-7} does not cause dispersion of pectic acid. That reaction takes place to some extent may, however, be shown by use of pectinic acid of low equivalent weight. When a dispersion of pectinic acid in 60% alcohol was allowed to absorb quinine and was then washed free of excess reagent with alcohol solution, the p_H of a 1% solution of the product was about 3.2 indicating $^5/_{10}$ neutralization. A similar experiment with pyridine, $K = 2.3 \times 10^{-9}$, gave a product with pectic acid which was not completely dispersible in the cold. The p_H was about 3.0. Excess pyridine causes complete dispersion of pectic acid. The obvious conclusion is that a difference in solubilities rather than in reactivities of the bases is responsible for the dispersion of pectic acid by pyridine but not by quinine.

With methyl glucamine, complete dispersion takes place. Solubility in alcohol is not so great as in the case of simple aliphatic amines.

The above compounds are prepared by common chemical procedures whereby basic and acid reacting materials are caused to combine. The exact procedure may vary with the different compounds because of their having greatly varying solubilities; for instance, such substances as are water soluble

may be added directly to a water suspension of finely divided pectic acid or to a water solution of the pectinic acid. In other cases where the organic reagent is not water soluble, it may be used in the form of a solution in a suitable organic solvent. Care must be taken not to have present any great excess of the base. Preferably, the total amount of base added should be slightly less than equivalent to the pectic or pectinic acid. A few specific examples of organic derivatives prepared are given below:

Triethanolamine Salt of Pectinic Acid.—To 45 Gm. of pectinic acid (about 450 eq. wt.) suspended in 80% alcohol are added 14.9 Gm. of triethanolamine as a 20% solution in alcohol. The triethanolamine solution is added slowly with constant stirring, and stirring is continued for 15 to 30 minutes after the addition has been completed. The liquid is filtered off, the product washed with alcohol, and dried.

n-Propylamine Salt of Pectic Acid.—To 44 Gm. of pectic acid (about 220 eq. wt.) suspended in 85% acetone are added 11.8 Gm. of *n*-propylamine as a 10% solution in acetone. The *n*-propylamine solution is added slowly with constant stirring, and stirring is continued for 15 to 30 minutes after the addition has been completed. The liquid is filtered off, the product washed with alcohol and dried.

Methylglucamine Salt of Pectic Acid.—To 22 Gm. of pectic acid suspended in water are added 19.4 Gm. of methylglucamine as a water solution with constant stirring. The resultant solution of methylglucamine pectate is poured slowly into two volumes of 95% alcohol. The precipitate produced is filtered off, washed with alcohol, pressed and dried.

SUMMARY

Purified pectins (free pectinic acids) and pectic acid are pictured as typical organic acids of high molecular weight. Differences in behavior result from the differences in equivalent weight which regulate the amounts of basic reagents combining with a pectin or with pectic acid. Typical titration curves for pectins are given. Reactions with either organic or inorganic bases are described.

Triethanolamine pectate is soluble in as high as 60% alcohol, and the propyl amine in 75% alcohol.

Other interesting properties of the organic base derivatives are suggested.

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Elkonite, A Colloidal Clay*

Properties, Actions and Possible Medicinal Uses of

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Elkonite is a naturally occurring clay, laid down in terrestrial beds near Elko, Nevada. When placed in water, it swells to many times its original volume, forming a firm jelly-like mass. Accordingly, it is a hydrophilic colloid with certain characteristics that set it apart from other similar materials commonly available in medicine. This paper presents the results of a study of the scope of possible clinical applicability and usefulness of Elkonite.

EXPERIMENTAL

Physical and Chemical Properties.—In the native state, Elkonite has the appearance of a light olive-green or greyish piece of soapstone. It is rather friable, so that pieces can be broken in the hand. Dispersed through it are small particles of sand or quartz comprising something less than 5 per cent of the total mass. When a piece of this clay is put in water, the surface softens and swells, taking on a gummy gelatinous consistency. If water is added in the proportion of 6 cc. for each Gm. of Elkonite, the swelling continues for several days until finally a homogeneous colloidal gel is secured, of the general consistency of library paste. This paste quivers when the container is struck a sharp blow, thus indicating an elasticity of an organized colloidal gel. Its physical state is not affected by normal temperature changes, the paste being stable under ordinary conditions as long as the evaporation of water is prevented.

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Less concentrated dispersions have the consistency of syrup; when still less concentrated, such as a 5 per cent concentration, the consistency and appearance resemble a greyish milk. In this low concentration, the clay solution readily passes through filter paper or cotton, leaving a sediment behind consisting of insoluble sand particles. The filterability can be used to free the Elkonite of all grit or particulate matter. Solutions can be concentrated by evaporating off the water by boiling, without destroying the colloidal nature of the clay. In fact, heating the clay to the temperature of softening of glass does not alter its characteristic physical state.

A fine non-gritty powder of Elkonite was prepared from a 4 per cent solution, which was filtered through coarse filter paper or cotton. The filtrate was collected and dried by evaporation, first on a steam-bath, and then at 110° C. for 24 hours. The resultant cake was ground in a mill to a fine powder and passed through a 100-mess sieve. This powder, or a 15 per cent paste made from it, has been used in all the tests or experiments to be described.

Chemically, Elkonite seems closely related to the general group of clays known as bentonites. However, it is much more colloidal than the bentonites known to us and comes from an area much farther west than the usual bentonite deposits. Quantitative analysis revealed the following elementary composition of the naturally occurring Elkonite:

Table I.—Composition of Elkonite

Moisture	12.83	per cent
Silica	52.70	per cent
Ferric oxide	1.96	per cent
Aluminum oxide	17.44	per cent
Calcium oxide	0.91	per cent
Magnesium oxide	3.63	per cent
Bismuth	0.011	per cent
Arsenic	Trace	
Ignition loss (moisture)	10.20	per cent
Lead, mercury, barium, fluorine, selenium and vanadium	None	

The presence of a trace of arsenic is not certain, since there was not enough in one-Gm. samples of the clay to give more than a suggestive mirror in the standard Marsh test. This suggests that any trace of arsenic which might be present is probably below the limit of physiological significance. The analytical results obtained indicate that Elkonite is essentially an aluminum magnesium silicate with small amounts of iron, calcium and bismuth present as impurities. Carbon or organic matter is not present.

The p_H of the Elkonite in 2 per cent solution was about 8.8 as judged by indicator solutions. A reading of 7.04 was obtained with a glass electrode, but this measurement may not have been reliable, since the Elkonite solution seemed to "poison" the glass electrode. In keeping with the slight alkalinity, the paste and solution taste mildy soapy, although not unpleasantly so. The material is moderately detergent, and has been used as a soap substitute under war-time conditions.

Adsorptive Power.—To determine whether the gel was an effective adsorbent for various materials, 2 experiments were performed on the adsorption of dyes. One gram. of Elkonite powder was added to 50 cc. of 1 per cent Congo red solution. After shaking for 24 hours, the colloid was separated by centrifugalization and the Congo red in the supernatant fluid estimated colorimetrically by comparison with an aliquot portion of the dye solution treated similarly, except for the Elkonite. Seventy-four mg. of the Congo red was found to be adsorbed by 1 Gm. of Elkonite. A similar test with methylene blue revealed that the Elkonite removed a 0.5-Gm. quantity of the dye so completely from the solution that less than 0.5 mg. remained in the supernatant fluid. Accordingly, definite adsorptive power of Elkonite for these dyes was demonstrated.

Because of the possible usefulness of a material of the type of Elkonite for internal administration as an adsorptive or protective agent in gastro-enteritis, colitis, gastric or duodenal ulcers, etc., it was desirable to know whether it would adsorb appreciable amounts of acid or alkali and thus modify the chemical reaction in the digestive tract. To compare its action with that of other generally available colloidal adsorbent or antacid powders, 9 different substances were tested in parallel for their efficiency in adsorbing acid and alkali. The materials tried were Elkonite, Trisomin (magnesium trisilicate), bentonite, fuller's earth, Norit (an adsorptive charcoal), kaolin, bismuth subcarbonate, aluminum hydroxide and Lloyd's alkaloidal reagent. The powders were all reduced to a fineness of at least 100 mesh. Then, 1 Gm. of each substance was placed in each of 2 bottles and a suspension made by the addition of 20 cc. of distilled water. The suspension was agitated for 24 hours on a mechanical shaker and the hydrogen ion concentration determined, using an overlapping series of 11 indicators with a p_H range between 3.1 and 10.5. To one series of bottles, 1.0-cc. quantities of 1 per cent hydrochloric acid were added until the p_H of the suspension reached 3.1. To the second series of bottles, 1.0-cc. quantities of 1 per cent sodium carbonate solution were added until the p_H reached 10.5. With each 1-cc. addition of acid or alkali, the bottles were agitated for 24 hours and the p_H redetermined. This experiment was repeated a second time with close agreement. Table II contains the average values obtained. It is seen that the best antacid was Trisomin. The remaining substances were arranged in descending order of efficiency as antacids as follows: fuller's earth, bismuth subcarbonate, Norit, Lloyd's reagent, Elkonite, bentonite, aluminum hydroxide and kaolin. In order of efficiency for neutralizing alkalies Lloyd's reagent was the best, then Trisomin, Elkonite, fuller's earth, kaolin, bismuth subcarbonate, aluminum hydroxide, Norit and bentonite. One Gm. of Elkonite required 2 cc. of acid to shift the p_H to

Table II.—p_H Changes with Increasing Quantities of 0.5 Per Cent Hydrochloric Acid or 1 Per Cent Sodium Carbonate Added to 1-Gm. Quantities of Various Insoluble Powders

Substance		Control	Volume Added (Cc.) 1	2	3	4	5	6	7	8	9	10	12	13	14
Elkonite	Acid	8.7	5.1	3.1	...	...	...	...	...	...	...	...	...	...	...
	Base	8.7	9.2	10.0	10.3	10.5	...	...	...	...	...	...	...	...	...
Trisomin	Acid	8.6	8.4	8.2	8.2	7.8	7.6	6.0	5.8	5.8	5.6	5.0	4.5	4.0	3.1
	Base	8.6	9.0	9.5	9.8	10.0	10.5	...	...	...	...	...	...	...	...
Bentonite	Acid	10.5	4.7	3.1	...	...	...	...	...	...	...	...	...	...	...
	Base	10.5	...	...	...	...	...	...	...	...	...	...	...	...	...
Fuller's earth	Acid	8.8	8.5	8.3	8.3	6.8	6.5	6.1	4.0	3.1	...	...	...	...	...
	Base	8.8	9.2	9.6	10.0	10.5	...	...	...	...	...	...	...	...	...
Norit	Acid	8.8	8.3	6.5	3.1	...	...	...	...	...	...	...	...	...	...
	Base	8.8	9.6	10.5	...	...	...	...	...	...	...	...	...	...	...
Kaolin	Acid	6.4	3.1	...	...	...	...	...	...	...	...	...	...	...	...
	Base	6.4	8.6	9.0	10.5	...	...	...	...	...	...	...	...	...	...
Bismuth subcarbonate	Acid	9.0	8.8	8.0	7.2	6.2	4.2	4.0	3.1	...	...	...	...	...	...
	Base	9.0	9.6	10.2	10.5	...	...	...	...	...	...	...	...	...	...
Aluminum hydroxide	Acid	9.2	3.1	...	...	...	...	...	...	...	...	...	...	...	...
	Base	9.2	9.0	9.5	10.5	...	...	...	...	...	...	...	...	...	...
Lloyd's reagent	Acid	9.0	6.6	5.0	3.1	...	...	...	...	...	...	...	...	...	...
	Base	9.0	8.8	8.8	8.8	9.0	9.0	9.4	9.8	10.0	10.5	...	...	...	...

Table III.—Viscosity of 1-Gm. Quantities of Various Insoluble Powders in 20 Cc. Water After the Addition of Successive Amounts of 0.5 Per Cent Hydrochloric Acid or 1 Per Cent Sodium Carbonate. (All Values Are Expressed as the Ratio of the Viscosity to That of Water)

Substance		Control Before Agitation	After Agitation	Volume Added (Cc.) 1	3	5	7	9	10	11	12	13	14
Elkonite	Acid	1.41	1.91	27.00	4.96	2.04	1.26	1.51	1.33	1.33	...	...	...
	Base	1.41	1.91	1.52	1.80	2.18	2.78	2.48	...	1.77	1.66	1.57	1.48
Trisomin	Acid	1.04	1.03	1.04	1.08	1.05	1.08	1.12	1.08	...	...	...	...
	Base	1.04	1.03	1.03	1.03	1.04	1.05	1.06	1.05	...	...	...	...
Bentonite	Acid	1.51	1.71	2.03	5.81	1.63	1.76	1.51	1.52	...	...	...	...
	Base	1.51	1.71	1.63	1.53	1.49	1.58	1.40	1.40	...	...	...	...
Fuller's earth	Acid	1.03	1.03	1.03	1.03	1.03	1.03	...	...	...	...	...	...
	Base	1.03	1.03	1.03	1.03	1.03	1.03	...	...	...	...	...	...
Norit	Acid	1.03	1.08	1.08	1.05	1.06	1.05	...	...	...	...	...	...
	Base	1.03	1.08	1.08	1.03	1.08	1.06	...	...	...	...	...	...
Kaolin	Acid	1.04	1.04	1.04	1.03	1.03	1.03	...	...	...	...	...	...
	Base	1.04	1.04	1.03	1.03	1.03	1.03	...	...	...	...	...	...
$BiOCO_3$	Acid	1.03	1.05	1.05	1.05	1.05	1.04	...	...	...	...	...	...
	Base	1.03	1.05	1.04	1.04	1.05	1.04	...	...	...	...	...	...
$Al(OH)_3$	Acid	1.05	1.03	1.03	1.03	1.05	1.04	...	...	...	...	...	...
	Base	1.05	1.03	1.02	1.02	1.03	1.02	...	...	...	...	...	...
Lloyd's reagent	Acid	1.03	1.03	1.03	1.05	1.03	1.05	...	...	...	...	...	...
	Base	1.03	1.03	1.04	1.06	1.11	1.18	...	...	...	...	...	...

3.1, and 4 cc. of the alkali to raise it to 10.5. Therefore, it was intermediate in the series as far as these properties were concerned.

Effect of Acid and Alkali upon Viscosity.—The changes in viscosity of the suspensions of the same 9 substances were studied after the addition of acid and alkali, since this would reveal changes in their colloidal state as the reaction was altered.

The viscosity of the different suspensions was determined by timing the emptying of a 10-cc. pipette filled with the suspension in question, and expressing the result in terms of the time required for water. All the determinations were performed at room temperature. Suspensions of the various powders in 5 per cent concentration were made up in the same way as for the p_H determinations, and each mixture was agitated for 24 hours after each addition of fluid. Table III presents the viscosity of the solutions divided by the viscosity of distilled water as unity.

Suspensions of Trisomin, fuller's earth, Norit, kaolin, bismuth subcarbonate and aluminum hydroxide showed no viscosity changes after the addition of alkali or acid. Lloyd's reagent increased slightly in viscosity after the addition of more than 3 cc. alkali. Bentonite suspensions increased sharply in viscosity to 5.81 after the addition of up to 3 cc. of acid, but the viscosity returned to the control value after 5 cc. There were no changes after the additions of base. In contrast, Elkonite-suspensions increased markedly in viscosity to 27.0 following the addition of acid, and to 2.78, of alkali. This change was so marked with 1 cc. of acid that the fluid suspension became nearly a gel. Almost as high a viscosity was attained with the 5 per cent suspension in the presence of acid, as was seen with a 15 per cent concentration at the normal p_H value. The viscosity was also above normal until 9 cc. of the alkali had been added. Therefore, Elkonite was much more hydrophilic in the presence of acid or alkaline reactions, such as those of the gastrointestinal tract, than were any of the other materials tested.

Effect on Gastro-Intestinal Motility in Man.—Two subjects took 0.8 Gm. Elkonite powder in capsules repeatedly to determine the effects, if any,

on the speed of passage of material through the gastro-intestinal tract. From 1 to 3 Gm. of medicinal charcoal were taken alone at breakfast and the time of its appearance in the feces recorded. In 8 such experiments, the average time of passage of charcoal was 11.8 hours. On 9 occasions the charcoal was taken together with 6 capsules, or 4.8 Gm. of powdered Elkonite. The average time of appearance of the charcoal was lengthened to 17.9 hours. In addition, the stools were in general of semi-solid or soft consistency, indicating a modification in physical state of the fecal material.

In one additional experiment, the progress of the same amount of Elkonite through the bowel was observed radiologically, using a small amount of barium sulfate in the capsules. There was no change in the rate of progress from that observed in normal individuals. Since the differences with the charcoals are also well within the normal range of variability, it was concluded, that, while the Elkonite may slow down the average rate of passage of material through the alimentary canal, it does not significantly affect the passage in any given individual beyond normal limits.

The main usefulness of Elkonite in the gastro-intestinal tract would seem to be as a colloidal demulcent, which could protect an irritated mucosa, and, by its gel-like consistency, give softness and bulk to the feces. In one patient, with a hyperirritable colon, the taking of one tablespoonful of the Elkonite paste with each meal for several months has been accompanied by appreciable relief of symptoms. This observation lends some support to the suggestion of its possible usefulness in this type of condition. In order to get the full effect of the Elkonite, it should be administered as the paste rather than the powder, since the powder may take 24 hours to swell to its maximum after it is brought in contact with the fluid of the intestinal tract.

Chronic Feeding Experiments in Rats.—The effect of continued ingestion of Elkonite was tested on rats, which were given the powdered material mixed with food. For this purpose, 9 albino rats weighing between 37 and 65 Gm. were divided into 2 groups and put into 2 cages. They were fed as a basic diet, the stock rat food used for our colony (1), to which was added 4 per cent barium sulfate to serve as a marker for radiologic studies. The food of the rats in one cage was medicated by adding 10 per cent of purified Elkonite powder. The food was supplied in the special all-glass feeders previously described (2), the amounts consumed being weighed at 2-day intervals. The rats were also weighed at the same time.

The average initial weights of the control rats were 59 Gm. On the 147th day, when the experimented were terminated, they weighed an average of 273 Gm. The Elkonite fed animals had an initial average weight of 54 Gm., and a final average of 250 Gm. Throughout the growth period, the weight curves of the two groups of rats ran closely parallel, the medicated animals falling within the range of the controls. Although the Elkonite rats averaged slightly lighter than the controls at the end of the experiment, there was no definite evidence of any adverse effect of the Elkonite on the nutrition of these animals, when it was fed to them for this five-month period.

The average food consumption of the control rats over the entire period was 17.4 Gm. daily for each rat. The Elkonite fed animals ate an average of 21.9 Gm. daily. When this latter value is corrected for the Elkonite present in the diet, the medicated rats ingested 19.7 Gm. daily, a value 13.2 per cent larger than the controls. Since the Elkonite rats gained no more weight than the controls, but rather slightly less, and had a larger food intake, the presence of the colloidal material interfered with the complete absorption of the food.

The distribution of the material in the alimentary canals of these rats was studied radiologically 6 times during the feeding experiment. At no time were there any evidences of alteration in motility, or abnormal distributions of the food mass, such as might be expected if balling or caking occurred. The Elkonite fed rats had clay colored stools of softer consistency than the controls, but there was nothing else that could be observed to differentiate them from the controls.

Finally, on the 147th day, all the rats were killed, and their gastro-intestinal tracts examined. In none of the animals were there any impactions or hardened masses, such as can occur when non-hydrophilic colloids are administered, and the stomach and intestines appeared entirely normal. The stomachs, small intestines and large intestines were separated, freed of contents and weighed. The average weight of the entire digestive tract in the controls was 10.28 Gm., or 3.7 Gm. per 100-Gm. body weight. The Elkonite group had an average total weight of 10.77 Gm. or 4.3 Gm. per 100-Gm. body weight. Therefore, the gastro-intestinal tracts were about 16 per cent heavier in the rats on the bulky diets than in the controls. When these differences were computed on the basis of body surface, the bulky diets produced an 11 per cent greater total weight per 100 sq. cm. of surface. The greater part of the increase was in the stomach, with a mass 19 per cent greater than the controls, while the small intestine was only 10 per cent, and the large intestine 6 per cent heavier, respectively. This effect of a bulky, high residue diet causing a work hypertrophy had been also observed by Addis (3), although he obtained somewhat greater increases with his more extreme diets.

Use as an Ointment Base.—A 15 per cent gel of Elkonite has a smooth, creamy consistency suitable for an ointment base. Any of the medicaments commonly used in the treatment of skin diseases may be incorporated with it. Ointments prepared with Elkonite as the base are homogeneous and smooth, and relatively small amounts are required for application. When rubbed on the skin, a dry film forms after 2 or 3 minutes, which holds the

medicament in contact with the skin-surface. Elkonite is not rubbed off by the clothing or bed linen, but can be readily washed off with water. Since it does not spread to the hair and produce sticky smears, Elkonite makes a more satisfactory base for scalp ointments than the bases now available. The tars and dyes, which are very sticky and messy when made up in the usual ointments, with Elkonite give clean, dry adherent films, which make the treatment cleaner and less irritating to the skin, and prevent soiling of clothing.

The Elkonite gel was tried as an ointment base in the treatment of 63 patients (25 males and 38 females) with the following dermatoses: dermatophytosis, contact, seborrhœic and atopic dermatitis, pityriasis rosea, lichen simplex chronicus, scabies, impetigo, acne and pruritus ani. Salicylic acid, ichthyol, ammoniated mercury, resorcin, sulfur, naftalan, crude coal tar and its derivatives, balsam of Peru, gentian violet and oil of cade were used. The combinations and concentrations of the drugs used were those ordinarily employed in the treatment of these dermatoses. No incompatibilities were observed. The ointments were well tolerated on the glabrous skin and scalp, both in adults and infants. The response of the various dermatoses to treatment were approximately the same as with the use of the same drugs in other ointment bases. However, the improvement in the physical characteristics of the ointments was a distinct advantage.

Ointments prepared with Elkonite base maintained their consistency despite changes in temperature for a period of more than three months. They appeared to change on standing only if left in open containers from which the water of the ointment could evaporate.

SUMMARY

1. Elkonite is a colloidal clay made up mainly of aluminum magnesium silicate, is hydrophilic and swells in water. In 15 per cent concentration, it forms a gel.

2. Addition of acid or alkali to fluid suspensions of Elkonite greatly increases the hydrophilic property as shown by increases in viscosity. Some of the acid or base is adsorbed on the colloid, as shown by p_H measurements.

3. The adsorptive power for dyes and ions and the gel-like nature of Elkonite indicate that it might be a useful agent for oral administration in gastro-intestinal disorders. Preliminary trials indicate that clinical effects can be obtained, as shown by alterations in intestinal motility, and relief of symptoms.

4. Continued administration for 5 months to rats of Elkonite in 10 per cent concentration in their diet had no demonstrable influence on the rate of growth, but resulted in a definite increase in food intake over that of a parallel series of control animals. Radiologic examination and direct inspection of the gastro-intestinal tract of these rats indicated normal conditions throughout the experimental period. However, there was a work hypertrophy of the digestive tract of the Elkonite fed rats amounting to an 11 per cent increase in weight over that of the controls, being more marked in the stomach than in the intestines.

5. The most promising clinical usefulness of Elkonite would appear to be as a gel of 15% strength to be used as an ointment base. This has the following advantages over bases in common use: dries on the skin and leaves an adherent film of the medicament which will not rub off, and does not stain or smear clothing, yet can be readily removed by washing with water. The advantage of a non-greasy or sticky ointment is especially marked in applying medication to the scalp and the pubic or perineal regions.

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Effect of Catalysts on Hydrogen Peroxide

Substances which catalyze the decomposition of hydrogen peroxide increase its germicidal effect against *Bacillus Coli*. It is reported that the addition of ferric or cupric sulfate increases the phenol coefficient of hydrogen peroxide 100 times. This effect is produced by the addition of 0.1 millimol of these salts to each ml. of peroxide.

Hydrogen Peroxide can be stabilized by the addition of certain agents, such as sulfuric acid. Urea, however, seems to be the most effective and satisfactory, although antipyrine serves almost as well.

A Chemical Investigation of the Seeds of *Glottidium Vesicarium* (Jacq.) Harper*

By P. A. Foote† and L. G. Gramling‡

Observations on *Glottidium vesicarium* (Jacq.) Harper were begun by the senior author in the fall of 1928. This weed, commonly called the Coffee Bean Weed, is found in the low moist places of Florida in large quantities. Hundreds of acres of it abound, in fact during one summer the larger portion of a four hundred acre tract in Hillsborough County was covered with it. It is common hearsay that Coffee Beans are toxic and that children, chickens and cattle have been poisoned by them. Investigation soon proved that many different beans of various species of plants have been given the name of Coffee Bean by the public. This has led to great confusion.

A description of *Glottidium vesicarium* (Jacq.) Harper as given by Erdman West, Mycologist of the Florida Agricultural Experiment Station, is as follows:

"Plant annual but somewhat woody, 1 to 3 M. tall, sometimes widely branched, often growing in large colonies in damp soil. Leaves, alternate pinnate, with 20 to 52 leaflets; leaflets elliptic, mucronate, 1.5 to 4 cm. long, dark green above, paler beneath. Flowers one to several in auxillary peduncles, 2 cm. or more long; corolla, yellow (or dark reddish purple in variety atrorubrum), about 1 cm. across; standard reniform, rounded; wings and keel angular; stamens 10, diadelphous, 0 to 1. Calyx 5-lobed, the lobes much shorter than the tube. Pods pendant, usually in pairs, ellipsoid, acuminate at both ends, 5 to 8 cm. long; outer wall dehiscent, inner wall remaining as a white papery envelope. Seeds 2, grey-brown, mottled. Flowers in summer and fall. Pods often remain on the dead stalks all winter. Coastal plain, North Carolina to Florida and Texas. Introduced from the West Indies."

The Texas variety seems to be the same. In the spring of 1931 Dr. S. E. Wolff, Botanist for the Texas Agricultural Experiment Station at Temple, Texas, sent us seeds which produced plants of the above description. We acknowledge his kindness with thanks.

EXPERIMENTAL

TOXIC PROPERTIES OF THE SEEDS

Rumored cases of children being poisoned by eating the beans could not be substantiated (1). Wolff (2) reported, "I, personally, have cooked the seeds and found them good." Matlack (3) advises that the roasted seeds are non-toxic to guinea pigs. In the fall of 1930 a senior student, C. Kazarian, working in our laboratory found 14 Gm. of the dried ground seed toxic for the cat. The material was mixed with twice its weight of salmon and fed to a cat which had been starved for 43 hours. Within two hours the following symptoms appeared: deep breathing, stupidity, nausea, violent vomiting which ended with yellow and green mucous, several watery stools with griping.

The intraperitoneal injection into rats of 3 cc. of extracts ranging in strength from 10 Gm. per 100 cc. to 100 Gm. per 100 cc., using 15 per cent and 50 per cent ethyl alcohol as menstrua, failed to give any noticeable systemic effects. A rat fed approximately five Gm. of the ground dried seed in twice that quantity of cheese did not show any noticeable ill effects.

The chief work on the toxicity of the seeds has been done by Emmel (4), in 1935, working at the Florida Agricultural Experiment Station. His work was on single comb white leghorn hens. He reported that 150 seeds, when force fed, is the minimum number which will kill a chicken. Investigation by him of the gross pathology, histopathology and blood-picture showed necrotic enteritis and necrosis of the lining of the bulbous portion of the gizzard. The hepatic cells showed fatty degeneration and the remaining parenchymatous organs and all involuntary muscles were in a state of cloudy swelling. There was a destruction of both erythrocytes and leukocytes.

ANALYTICAL

The literature failed to reveal any work of this nature. A thesis by Weedon (5), in 1923, is all that could be found. He reported no alkaloids nor glucosides and so directed his attention largely to the fatty oil. This he obtained by ether extraction in a yield of 6.1 per cent. It had the following properties: specific gravity 0.933 at 35° C.; refractive index 1.480, corrected to 15.0° C.; saponification value 193.0; acid value 3.2; iodine value 132.0. The fatty acids had an iodine value of 138.5. Weedon found the oil to have excellent drying properties and to be a good substitute for linseed oil in paints. He estimated the protein of the seeds to be about 21 per cent.

Fatty Oil.—Our work began by collecting 1 Kg. of the seeds near Gainesville, Florida in the fall of

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1930. Preliminary investigation showed no alkaloids nor glucosides to be present. A saponin was indicated by all the well-known tests. Previous to attempting its isolation the fatty oil was investigated. Eight hundred twenty-nine grams of ground seeds dried at 105° C. yielded 3.39 per cent of fatty oil by petroleum ether extraction. The chemical and physical properties checked well with those found by Weedon. A sample of the oil stored in a well filled bottle in diffused light has become very viscid, indicating polymerization. This, of course, is to be expected from the high iodine value of the oil.

Saponin.—After the above petroleum ether extraction, the seeds were extracted with 80 per cent alcohol. When this was distilled off 8.5 Gm. of a syrupy residue remained. It gave a negative test for saponin. It had an odor of caramel and gave the osazone of dextrose. After standing for several months over concentrated sulfuric acid it partially crystallized. Apparently the saponin had hydrolyzed during extraction and is but another instance illustrating the well known difficulty of isolating these bodies in a pure condition Repeated efforts, using also methyl alcohol extracts from three Kg. of seeds, failed to produce a pure saponin; therefore, the following efforts were made to get the sapogenin or product of hydrolysis other than the sugar.

Sapogenin.—Using the procedure of Liang, and Noller (6), the dried alcohol-water extract was dissolved in just sufficient methyl alcohol containing 5 per cent HCl and refluxed continuously for forty-eight hours. The dark brown precipitate which formed was washed with water, dried in air and extracted two hours in a Soxhlet extractor using carbon tetrachloride. The solvent was allowed to evaporate at room temperature, leaving a cream-colored material. Hot methyl alcohol was used to dissolve all the material that it would and normal isopropyl alcohol was used to dissolve the remainder.

The methyl alcohol solution was chilled in an ice-bath and some material separated which on drying in air was a light tan color and glistened. The mother liquor was concentrated and again chilled producing a second crop of material. The quantities were so small that further purification was not attempted, but the material appeared crystalline. Melting points of these two portions were not very sharp, ranging from 48° C. to 54° C.

The normal isopropyl alcohol solution was also chilled and a white crystalline material separated. This was washed with water, dried and the melting point determined as 47° C., uncorrected. Further crystallization from the mother liquor gave crystals whose melting points checked closely. The crystals gave negative tests for sulfur, nitrogen and halogens. Since the quantity of material was so small no further data could be obtained.

It is indicated that an alcoholic-aqueous extract of these seeds yields, upon hydrolysis with hydrochloric acid, one or more sapogenins and it is hoped that subsequent work with larger quantities will enable their identification.

SUMMARY

The available data on the toxicity of *Glottidium vesicarium* (Jacq.) Harper have been pointed out and the previous work on the seeds, consisting principally of an investigation of the fatty oil, has been reviewed. The data on the fatty oil have been checked and negative tests for alkaloids and glucosides have been obtained. Positive tests for saponin have been obtained but the saponin appears to be hydrolyzed during extraction, and thus far attempts at isolation have failed. One or more hydrolysis products have been obtained by refluxing an alcoholic-aqueous extract with 5 per cent HCl.

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Crystalline Xanthophyll from Wheat Germ

*By O. Gisvold**

While attempting to isolate the tocopherols by new methods, a crystalline xanthophyll was obtained from the nonsaponifiable portion of wheat germ. A small amount of an allophanate of one of the tocopherols was also isolated.

EXPERIMENTAL

Twenty-five pounds of wheat germ were extracted with hot Skelly-solve B in a Barnsdahl continuous extraction apparatus. There was obtained 1200 Gm. of oil which was saponified in the usual manner. The nonsaponifiables were separated from the soap by continuous extraction with

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Skelly-solve B. The nonsaponifiable material thus obtained was dissolved in alcohol and most of the sterols were removed from this solution by fractional crystallization.

Attempted Isolation of the Tocopherols by Means of Their Lead Salts.—An alcoholic solution of normal lead acetate was added to the alcoholic solution of the nonsaponifiable material. Precipitation did not occur, even after concentration or cooling in the ice chest. The alcohol was completely removed on the steam bath and the residue treated with Skelly-solve B. The material insoluble in this solvent was suspended in alcohol and decomposed by hydrogen sulfide. A very small amount of a semi-solid was obtained.

When basic lead acetate was substituted for normal lead acetate the same results were obtained.

Attempted Isolation of the Tocopherols by Means of Their Barium Salts.—Essentially the same procedure was followed as outlined above with the exception that methanol was used as the solvent and a methanol solution of barium hydroxide as the precipitant. The results were negative.

Attempted Isolation of the Tocopherols by Extraction of a Skelly-solve B Solution of the Nonsaponifiables by Means of Aqueous and Alcoholic Sodium Hydroxide.—The nonsaponifiable material was dissolved in Skelly-solve B. It was extracted with 5, 10 and 20 per cent solutions of aqueous sodium hydroxide. When these solutions were neutralized with phosphoric acid and extracted with ether, a very small amount of a semisolid material was obtained. The nonsaponifiable portion was then extracted with an approximately 25 per cent solution of sodium hydroxide in 25 to 33 per cent alcohol. The alkaline extractive was colored and, upon decomposition with phosphoric acid and extraction with ether, yielded a small amount of an oil. From this oil an exceedingly small amount of an allophanate, m. p. 145° C., was obtained. This allophanate failed to give the color reactions characteristic of sterols and by virtue of its melting point was thought to be one of the tocopherols.

Crystalline Xanthophyll.—The petroleum ether solution that had been extracted with an alcoholic solution of alkali was allowed to stand in the ice chest for some time. Rosettes of garnet red leaflets separated from this solution. They were collected upon a fritted glass filter, washed with Skelly-solve B and dried. They melted at 175° C. and exhibited an absorption max. at 475, 450 in alcohol. A portion of the material when dissolved in Skelly-solve B would partition itself into 85 per cent methanol. A solution of the pigment in chloroform gave a bluish violet color when carefully stratified with strong sulfuric acid.

SUMMARY

Crystalline xanthophyll was obtained from the nonsaponifiable portion of wheat germ oil while attempting to isolate the tocopherols by new methods.

Colormetric Determination of Thiamin Chloride in Certain Pharmaceutical Preparations

*By M. E. Auerbach**

Recently, Prebluda and McCollum (1) have described in detail a chemical reagent for thiamin chloride. Using the same reagent Melnick and Field (2) have developed procedures which make possible a quantitative determination in a variety of substances. The principle elaborated by these investigators is that thiamin will couple with diazotized *p*-amino acetophenone to produce a purplish red compound. This compound is insoluble in water, but is readily soluble in various organic solvents, thus providing a colored solution suitable for comparison with standards. Melnick and Field prefer to use xylol as the color solvent. We use toluol, which is equally serviceable. For the analysis of thiamin dissolved in a simple, colorless menstruum, it is unnecessary to extract the colored compound from its mother liquor. In such cases, we simply add enough isopropanol to dissolve the color, and proceed directly to the quantitative comparison with a reference solution.

A certain drawback appears in the work quoted, in that at least 12 hours are stated to be necessary for the development of the maximum color intensity. During the past two years, we have employed a modification of the Prebluda-McCollum test which permits a complete analysis of thiamin in certain pharmaceutical products within 15 to 30 minutes. The same method, with minor alterations, has served for the determination of the stability and incompatibilities of thiamin chloride under various conditions and in various media.

EXPERIMENTAL

Conditions for the Analysis.—A quantity of test sample is taken which will yield as nearly as possible 100 micrograms of thiamin. A standard solution is made by dissolving exactly 50 mg. of pure dry thiamin chloride (or an equivalent calculated from actual analysis) in 500 cc. of 50% (volume) ethanol containing one drop of 4*N* HCl. This standard is stable at room temperature for several months, but

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to be on the safe side, we prepare a fresh standard every four weeks.

Reagents:

(1) 0.03% *p*-amino acetophenone (Eastman Kodak Co.) in 0.2*N* HCl. This solution is stable indefinitely.
(2) 0.1% sodium nitrite. Prepare fresh daily.
(3) Normal NaOH and 2.5*N* NaOH.
(4) Ethanol, 50% (volume).
(5) Toluol.
(6) Isopropanol.
(7) 4*N* HCl.

The solution referred to as "diazo solution A" is prepared as follows: In a small flask mix 10 cc. of the amino acetophenone solution and 2 cc. of the sodium nitrite solution. Let stand in ice water for at least two minutes. Just before the solution is to be used, add 3 cc. of normal NaOH and mix. "Diazo solution B" is similarly prepared, 2.5*N* NaOH being used instead of normal NaOH.

COURSE OF THE ANALYSIS

A. Ampule Solutions.—Dilute the vitamin solution with distilled water, so that each cc. is estimated to contain about 100γ. In a clean test-tube with a 10-cc. calibration mark, take 1 cc. of this dilution, add 2 cc. of 50% ethanol and place the tube in a water-bath kept at 60° C. After a minute, add one cc. of diazo solution A. Mix well and let stand at 60° C. for another 2–3 minutes. Cool, add one drop of 4*N* HCl, then add isopropanol to the calibration mark, mix, transfer to a colorimeter tube and compare with the reference solution. The reference solution is prepared by taking one cc. of distilled water in a tube, then adding one cc. of the standard thiamin solution, one cc. 50% ethanol and one cc. diazo solution A. in the manner already described.

B. Thiamin Chloride Elixir.—The elixir in question contains about 17 mg. thiamin per 100 cc. If 6 cc. of elixir are mixed with 4 cc. of water, the mixture will contain close to 100γ per cc. Take one cc. of this dilution in a clean test-tube, add 3 cc. 50% ethanol, warm to 60° C. in a water-bath, then add 2 cc. of diazo solution B. Keep at 60° C. for another 15 minutes. Cool, acidify with a few drops of 4*N* HCl and transfer to a small separatory funnel. Dilute with water to 25–30 cc., then add exactly 8 cc. toluol and shake. The water insoluble, colored vitamin derivative is readily taken up by toluol, especially from acid solutions. Add 3 cc. of 2.5*N* NaOH, shake, allow to separate, discard the aqueous layer, wash the toluol layer once with distilled water, then transfer to a centrifuge tube. Centrifuge for a few minutes (until perfectly clear and bright) and compare with the reference solution. The latter is prepared by diluting 6 cc. of vitamin-free elixir with 4 cc. water. To one cc. of the dilution add one cc. of standard thiamin solution, then 2 cc. 50% ethanol, and continue as given above. The vitamin-free elixir can be prepared by shaking out the original elixir with a suitable adsorbent, such as Decalso (2), (3).

C. Tablets Containing Thiamin Chloride.—Thoroughly pulverize ten tablets. Weigh out an amount which will contain about one mg. thiamin, and transfer to a test-tube with a 10-cc. calibration mark. Fill to the mark with 50% ethanol, and let the tube stand in a 60° C. water-bath for ten minutes, with occasional gentle mixing. Cool, and if necessary add alcohol to bring the level back to the mark. Mix well and centrifuge until clear. To one cc. of the clear centrifugate add 1 cc. 50% ethanol and 1 cc. water, warm to 60° C. and add 1 cc. diazo solution B. Keep at 60° C. for another 2–3 minutes, cool, acidify with 4*N* HCl, then extract with toluol as described in section B. Compare the colored toluol solution with a reference solution prepared from a mixture of 1 cc. water, 1 cc. 50% ethanol, 1 cc. standard solution and 1 cc. diazo solution B.

GENERAL DISCUSSION

When 100 micrograms are taken for a standard reference solution, and the quantity in the test sample is in the range 90–110 micrograms, the error of the method is not greater than ±3%. The error increases in a fairly definite way as the discrepancy between the test quantity and standard quantity increases.

We have found it possible to determine thiamin chloride in such varied solvents as milk, wine, orange juice, mineral oil, propylene glycol, alcohol, glycerol and sugar syrup and in aqueous or/and alcoholic solutions also containing phenol, chlorbutanol, benzoates, citrates, bromides, iodides, lactates, salicylates, phosphates, glycerophosphates, ferric ammonium citrate, vitamins A and D_2, riboflavin, ascorbic acid, barbiturates, amidopyrine, pepsin, liver extract. In some cases a simple preliminary treatment was necessary. For example, solutions containing liver extract are best handled by diluting with three or four parts of methanol, centrifuging off the alcohol-insoluble constituents, and taking a convenient aliquot of the centrifugate for the determination. The presence of ascorbic acid in the test sample involves minor additional treatment which we hope to describe in the near future.

With few exceptions, it is necessary to run a blank of the same composition as the test sample, since many substances affect the

intensity and shade of the color produced. Within our experience, however, no thiamin-free substance has yielded a positive reation. Mixtures of unknown composition are readily de-vitaminized by shaking with Decalso at p_H 4.0–4.5 (2), (3).

For good results, it is important to plan the quantities of reagents used so that the coupling reaction takes place in a mixture containing 20–25% ethyl (or methyl) alcohol.

The specificity of the Prebluda-McCollum color test depends largely on the fact that the colored thiamin derivative is insoluble in both acids and alkalis. We always extract the color from an acid medium, to avoid the annoyance of the emulsions which tend to form when shaking alkaline solutions. We then wash the colored toluol solution with sodium hydroxide solution, to remove foreign colored substances containing acidic groups such as carboxyl or phenol hydroxyl.

SUMMARY

A simple and rapid method for the quantitative estimation of thiamin chloride has been described. The method is based upon the Prebluda-McCollum reaction. It has successfully been applied to the analysis of mixtures of pharmaceutical interest, containing as little as twenty micrograms of thiamin chloride per Gm. of sample.

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ADDENDUM

Colorimetric Determination of Thiamin Chloride in the Presence of Ascorbic Acid.— In the foregoing paper, several examples were given of a colorimetric determination of thiamin chloride in pharmaceutical products. It was stated that the presence of vitamins A, B_2 and D causes no difficulty with the method, but that the presence of vitamin C necessitates a special pre-treatment. Both ascorbic acid and its oxidation products prevent the development of the colored thiamin derivative upon which the determination depends. It was discovered that the addition of calcium ion (magnesium and barium are partially effective) to the mixture of thiamin chloride and oxidized ascorbic acid adequately prevented interfering reactions. The mechanism by which this is accomplished is not yet clarified.

The example given below presents details of the modified method. The product chosen for description consists of a suspension of vitamins A, B_1, B_2, C and D in a fat.

Method:. Transfer about one Gm. of the thoroughly mixed fatty mass to a tared 50-cc. Erlenmeyer flask. Add 5 cc. monochlorobenzene and then, from a burette, 10 cc. of $N/10$ HCl for each mg. of thiamin chloride estimated to be in the sample taken. Stopper and shake the flask thoroughly for five minutes, then centrifuge the contents until the aqueous layer is perfectly clear. The fatty material and vitamins A and D are retained by the monochlorobenzene. Thiamin chloride, riboflavin and ascorbic acid are all quantitatively extracted by the acid water.

Take one cc. of the aqueous extract in a clean test-tube, add 1 cc. 50% ethanol, then, drop by drop, strong fresh bromine water until the color is permanently a deeper yellow than the original solution. Add one drop of 4% sodium salicylate solution to take up excess bromine, then 2 cc. of 50% ethanol and finally 1 cc. of approximately $N/1$ $CaCl_2$. Warm in a water-bath at 60° C. and add 2 cc. of freshly prepared diazo solution.

(The diazo solution is prepared by mixing 10 cc. of 0.03% *p*-amino acetophenone in $N/5$ HCl with 2-cc. 0.1% $NaNO_2$. Place in an ice-bath for at least 3–4 minutes, then add 3 cc. of 10% NaOH just before using.)

Allow the tube to stand at 60° C. for 10–15 minutes, cool, acidify with a few drops of $4N$ HCl, add 8 cc. toluol, shake well and transfer to a small separatory funnel. Discard the aqueous layer, then wash the colored toluol with (1) about 15 cc. water; (2) about 15 cc. 4% NaOH; (3) about 15 cc. water. Transfer the colored toluol to a small centri-

fuge tube, spin until perfectly clear and compare in the colorimeter with a standard solution.

The standard is prepared by mixing 1 cc. of $N/10$ HCl with 1 cc. of 50% ethanol containing 100γ B_1. To parallel conditions in the actual analysis, we add to this mixture one drop of bromine water, one drop of 4% sodium salicylate, then proceed exactly as with the sample.

A Modification of the Agar Cup Method Suitable for the Estimation of the Fungistatic Action of Powders and Ointments*

By Arthur E. Meyer

In experiments of estimating the penetrating and growth inhibiting action of compounded products on *Trichophyton*, we found the agar cup test not quite satisfactory, for which reason a modification was worked out, which we would call the "l ne test."

EXPERIMENTAL

The substances investigated were oxyquinoline benzoate and malachite green, as such and incorporated in powder and ointment respectively. The powder contained 0.3% oxyquinoline benzoate and 3% boric acid in pure talcum. The first ointment contained 0.3% oxyquinoline benzoate and 3% boric acid in a 20% lanolin-water basis, the second 0.2% oxyquinoline benzoate with 0.01% malachite green with 3% boric acid and the same basis.

The fungicide properties of both active substances had previously been tested by the method of Burlingame and Reddish, showing that the 0.1% solution of oxyquinoline benzoate destroys *Epidermophyton interdigitale* after 15 minutes, 0.1% malachite green after 5 minutes, whereas *Trichophyton rosaceum* was not destroyed by either substance at the concentration used within 30 minutes.

The line test was performed as follows. A moderately thick plate of Sabouraud's medium was poured in Petri dishes, and with a sterile knife a 2 mm. wide strip was cut out across the plate through the center. The strip is easily lifted out with a sterile spatula. A sterile glass rod laid across the dish may serve as a guide to the knife. It is advisable to incubate the plate for a few days to make sure that no contamination has taken place. During that time the dish should be sealed with parafilm to prevent drying out.

Three days cultures of *Trichophyton rosaceum* and 5 days cultures of *E. interdigitale* were used. For that purpose a few cc. of sterile broth was poured on the fungus surface and the latter thoroughly suspended by rubbing with a sterile cotton swab. The same swab was used immediately to wipe the suspension over the whole surface of the divided plates. The wiping will occasionally cause slight scratches on the smooth surface of the agar, which are an inconvenience if photographs are to be taken, but do not influence the value of the test. In testing the

Fig. 1.

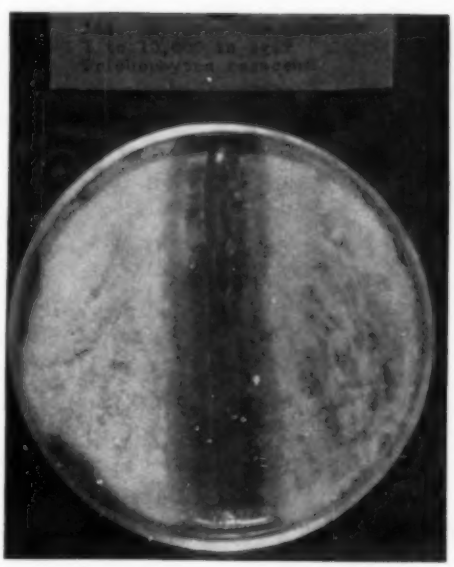

Fig. 2.

* From the Research Laboratories of the Maltine Company, Brooklyn, N. Y.

fungicides themselves, they were poured into the strip as a 0.01% solution in liquefied agar, at a temperature slightly above congealing point. The powder was mixed with water to a thick paste and applied, similarly to the ointments, by squeezing a strip out of a narrow hole, *i. e.*, using at in tube. The strip was placed in the canal in the agar and gently pressed down with a sterile spatula.

The fungi grow within due time in parallel lines to the center strip and give an illustrating picture of the efficacy and diffusion of the fungicide. There is no difficulty in inverting the dishes for incubation; even the powder stays perfectly within the strip.

From the experiments reported it may be concluded that *E. interdigitale* is too sensitive to be used at the concentration of the drugs as employed.

Fig. 3.

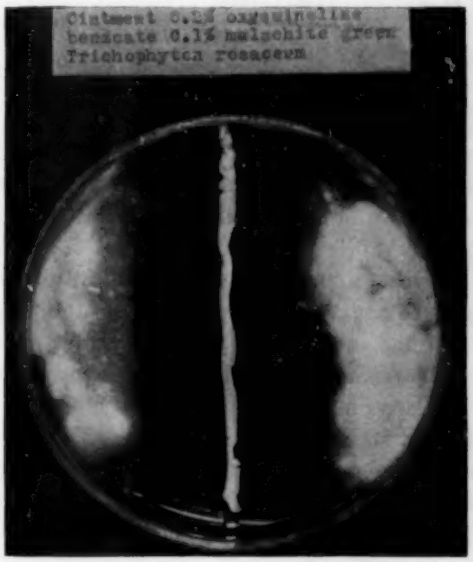

Fig. 4.

Table I.—Readings on Line Tests

No. of Plates	Material Used	Type Organism	Readings
1	0.01% Malachite green agar	*Trichophyton rosaceum*	After 2 days' growth at $1^1/_2$-cm. distance on both sides, after 3 days at 1-cm. distance. No further narrowing of sterile zone
2	0.01% Malachite green agar	*Trichophyton rosaceum*	After 4 days' growth at $1^1/_4$-cm. distance on both sides
3–4	0.01% Malachite green agar	*E. interdigitale*	After 5 days' parallel growth at $2^1/_2$-cm. distance on both
5–6	0.01% Oxyquinoline benzoate agar	*Trichophyton rosaceum*	After 2 days' growth to 1-cm. distance, after 3 days 0.7-cm. distance, after 4 days 0.2-cm. distance; no further change
7–10	0.01% Oxyquinoline benzoate agar	*E. interdigitale*	After 6 days varying from 1 to $2^1/_2$-cm. distance
11–12	Powder 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 2 days' growth at $2^1/_2$–3-cm. distance, after 3 days 1–2-cm. distance
13–14	Powder 0.3% Oxyquinoline benzoate	*E. interdigitale*	No growth
15	Powder 0.3% Oxyquinoline benzoate	*E. interdigitale*	After 7 days two small cultures at 3-cm. distance
16	Ointment 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 4 days' growth at 2-cm. distance, no further change
17	Ointment 0.3% Oxyquinoline benzoate	*Trichophyton rosaceum*	After 7 days' growth on one side only at 3-cm. distance
18–19	Ointment 0.3% Oxyquinoline benzoate	*E. interdigitale*	No growth
20–22	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*Trichophyton rosaceum*	After 5 days' maximum growth at 2–$2^1/_2$-cm. distance
23	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*E. interdigitale*	No growth
24	Ointment 0.2% Oxyquinoline benzoate 0.1% Malachite gr.	*E. interdigitale*	After 5 days very small colony at edge of dish on one side only

The method described has the advantage that the surface of contact between disinfectant and medium is considerably increased and shows better the evenness of action than is the case with the agar cup method. It also imitates the use of thin layers as applied for practical purposes.

Rapid Staining Methods in Plant Histology

By Robert S. McLean and Edward J. Ireland†*

The routine methods for the differential staining of plant tissue for histological study in use to-day require from three to twenty-four hours or more. McClung (1929) and Chamberlain (1932) recommend for routine work a primary stain of Safranin for three to twenty-four hours at room temperature, and a counterstain of hæmatoxylin for one-half to six hours. Chamberlain (1932) recommends six hours or more for a primary stain of Malachite Green or Methylene Blue, and one-half to one minute for counterstaining with Eosin or Erythrosin. These procedures are typical with respect to the time required to obtain an effective differential stain.

Obersteiner (1878) used a solution of carmine over a bath of boiling water for staining nerve cells. Bacteriologists use heat for staining bacterial spores and such organisms as *Mycobacterium tuberculosis* which "take" a stain slowly. By the use of heat in the "acid-fast" technique, the tuberculosis organism is stained in four or five minutes where twelve to twenty-four hours at room temperature are required.

Since heat is applicable in bacteriological staining, it seemed logical to assume that it would also be effective in botanical staining. Therefore the use of heat was investigated and successfully used in the following methods.

EXPERIMENTAL

To avoid the numerous transfers through increasing and decreasing concentrations of the alcohol, 95 per cent ethyl alcohol was used as the solvent for the stains in all cases except for one of the counterstains, Methyl Blue. For this stain, 80 per cent ethyl alcohol was used since the dye was very slightly soluble in the 95 per cent alcohol. The few stains which have a standardized formula were prepared with the directed solvent.

Paraffin sections of ten to twenty microns thickness fixed to slides with Mayer's albumin fixative were used throughout the series of experiments.

METHOD I

The first method which gave consistently good differentiation and showed considerable saving of time was conducted as follows:

1. Two to four drops of the primary stain (or enough to cover the sections) were placed on the slide, the solvent ignited and allowed to burn off completely.
2. The slide was washed briefly but thoroughly in 95 per cent ethyl alcohol. This was best accomplished by agitating the slide in the alcohol contained in a deep beaker or Coplin jar. The washing removed the excess stain from the slide and partially removed the primary stain from the cellulose tissue.
3. Four to six drops (or enough to flood the sections) of the counterstain were placed on the slide and allowed to act for ten to thirty seconds as required (see Table I). For the slower acting counterstains, Methyl Blue and Orange G especially, the solvent was burned off as in (1). With woody sections even the faster acting counterstains gave best results when flamed.
4. The slide was again washed in 95 per cent ethyl alcohol as in (2), cleared and examined.

Table I.—Relative Approximate Time Required for the Various Counterstains for the Above Process

Group I 5–10 Seconds	Group II 10–20 Seconds	Group III 20–30 Seconds
Erythrosin	Eosin Y	Orange G
Eosin B	Picric Acid	Methyl Blue
Orange I	Acriflavine	Methyl Red
Acid Fuchsin	Congo Corinth	
	Bismarck Brown	
	Hæmatin	

The chief difficulty encountered in this technique was the manipulation of the counterstains. The time required to obtain the proper degree of differentiation varied with the counterstain used and also with the nature of the sections, but this variation was only a matter of a few seconds. For example, Acid Fuchsin used on leaves or on herbaceous stems required only about 5 to 10 seconds, Eosin Y required 10 to 15 seconds and Methyl Blue about 20–30 seconds. On more woody sections these time ranges increased about 5 to 10 seconds. The lengths of time as classified in Table I were found to be only approximate. Since the time during which the counterstain was allowed to act was, in most cases, so very short, accurate timing was impossible.

Perhaps the best method of handling the counterstain is to flood the sections with the counterstain, allow it to act for five to ten seconds, wash briefly in

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the alcohol and examine the sections microscopically while still wet with the alcohol. If the desired degree of differentiation had not been obtained, the counterstain was applied again and allowed to act for five to ten seconds longer, the slide washed and examined again. This process was repeated until the proper differentiation was obtained.

Another method of handling the counterstain found to be of value in staining fairly large sections, such as cross or longitudinal sections of roots and stems measuring more than about 5 mm. in diameter or width, was as follows: The slide was held over a piece of white paper after the primary stain had been applied, the sections were flooded with a few drops of the counterstain and allowed to act until the color of the sections changed from the color of the primary stain to that of the counterstain. Then the excess stain was poured off, the slide was washed and examined. This method almost invariably stopped the action of the counterstain at the point where differentiation was good.

It was found that if the counterstain was allowed to act too long the primary stain was retracted from the lignified tissue and replaced by the counterstain as is the case in the routine staining methods. However, when this happened using this "flaming" technique the primary stain could be applied again as indicated above. The sections did not need to be decolorized before restaining.

The handling of the counterstain was easily mastered after a few attempts, the author often being able to estimate accurately the time necessary for a given counterstain to act. Usually only three or four slides were necessary to establish the time range for each counterstain.

The following stains have proved to be suitable for lignified tissue, cork and cuticle:

1.	Victoria Green	(du Pont)
2.	Malachite Green oxalate	(National Aniline & Chem. Co.)
3.	Brilliant Cresyl Blue	(National Aniline & Chem. Co.)
4.	Brilliant Green	(National Aniline & Chem. Co.)
5.	Janus Green	(National Aniline & Chem. Co.)
6.	Thionin	(National Aniline & Chem. Co.)
7.	Azure II	(National Aniline & Chem. Co.)
8.	Methylene Blue	(U. S. P. medicinal)
9.	Safrainie A	(Coleman & Bell)
10.	Rhodamine B	(Coleman & Bell)
11.	Hæmatin	(Coleman & Bell)
12.	Basic Fuchsin	(Coleman & Bell)
13.	Gentian Violet	(Coleman & Bell)

Of these, Hæmatin gave somewhat poor results. This was probably due to the fact that it imparted only a very indistinct color to the tissue, and this was easily replaced by, and difficult to differentiate with, any of the counterstains. Basic Fuchsin left a rather dense film over the sections which was hard to differentiate properly.

The following have proved to be applicable as counter-stains:

1.	Erythrosin	(Coleman & Bell)
2.	Eosin Y	(Coleman & Bell)
3.	Eosin B	(Coleman & Bell)
4.	Orange G	(Coleman & Bell)
5.	Orange I	(Coleman & Bell)
6.	Acid Fuchsin	(Coleman & Bell)
7.	Congo Corinth G	(Coleman & Bell)
8.	Hæmatin	(Coleman & Bell)
9.	Methyl Blue[a]	(Coleman & Bell)
10.	Picric Acid	(Merck)
11.	Bismarck Brown	(Merck)
12.	Acriflavine	(U. S. P. medicinal)
13.	Methyl Red	(Eastman Kodak & Chem. Co.)

[a] Saturated soln. in 80 per cent ethyl alcohol, due to low solubility in 95 per cent.

Of these, Congo Corinth, Methyl Red and Hæmatin gave poor results, probably because they gave a very indistinct color, hard to obtain differentiation without retracting the primary stain, and easily removed in the final washing process.

All stains used in this method were in 1 per cent solution except Acid Fuchsin, Orange I and these dyes were less than 1 per cent soluble. Acid Fuchsin and Orange I were used in a 0.5 per cent solution due to the fact that higher concentrations acted too fast to be readily controlled. Those dyes which were less than 1 per cent soluble were used in saturated solution. As stated above, 95 per cent ethyl alcohol was the solvent throughout, except for Methyl Blue which was dissolved in 80 per cent ethyl alcohol due to poor solubility in the 95 per cent.

Perhaps the best combinations of stains observed for use on leaf sections were Methylene Blue counterstained with Eosin or Erythrosin; Victoria Green with Picric Acid; and Safranin A or Rhodamine B with Methyl Blue. The best combinations observed for use on stem and root sections were: Methylene Blue counterstained with Eosin, Erythrosin or Orange G; Malachite Green with Acid Fuchsin or Orange I; and Safranin A with Methyl Blue.

METHOD II

The next step which seemed logical in the investigations of possible rapid staining methods was to attempt to combine two stains, a primary and a counterstain in a single solution. This would further reduce the time necessary to obtain an effective differential stain. After many attempts, this was accomplished, thereby obtaining a good differential stain using only two simple operations, and reducing the time to about fifteen to thirty seconds for the complete operation.

The solution used in this double stain technique was prepared by mixing a solution of a primary with one of the counterstains used in Method I. The proportions were varied until a satisfactory mixture was found. The first combinations found indicated that approximately 10 parts of the counterstain were needed for 1 part of the primary stain. Other combinations indicated that while this proportion was generally true, there was a variation necessary for some pairs of stains. Later it was found that

some of the more common double stains used in blood stainjng were also applicable to the differential staining of plant tissue. Wright's, Giemsa's and Jenner's stains were the ones tried, and these were satisfactory.

The technique in this method was identical with Steps 1 and 2 of Method I; the sections were covered with the mixture of the stains, the solvent ignited and allowed to burn off, the slide washed briefly but thoroughly in 95 per cent ethyl alcohol.

In this technique, it was found that the time during which the sections were washed varied with the various stain combinations and with the nature of the sections. With some stain, woody sections required a longer washing process than did herbaceous sections. Generally, the time for the washing process is about five to fifteen seconds.

The following proved to be suitable as double stains:

1.	Malachite Green (1% soln. in 95% ethyl alcohol)	0.1 cc.
	Acid Fuchsin (0.5% soln. in 95% ethyl alcohol)	1.0 cc.
2.	Victoria Green (1% soln. in 95% ethyl alcohol	0.1 cc.
	Acid Fuchsin (0.5% soln. in 95% ethyl alcohol	1.0 cc.
3.	Basic Fuchsin (1% soln. in 95% ethyl alcohol)	0.1 cc.
	Methyl Blue (satd. soln. in 80% ethyl alcohol)	1.0 cc.
4.	Safranin A (1% soln. in 95% ethyl alcohol)	0.3 cc.
	Orange G (satd. soln. in 95% ethyl alcohol)	2.0 cc.
5.	Wright's Stain (standardized formula) (methyl alcohol solution)	
6.	Giemsa's Stain (standardized formula)	
7.	Jenner's Stain (powder, satd. soln. in 95% ethyl alcohol)	
8.	Wright's Stain (powder, satd. soln. in 95% ethyl alcohol)	
9.	Azure II—Eosin[a] (powder, satd. soln. in 95% ethyl alcohol)	
10.	Methylene Blue (1% soln. in 95% ethyl alcohol)	0.2 cc.
	Acid Fuchsin (0.5% soln. in 95% ethyl alcohol)	1.0 cc.
11.	Methylene Blue (1% soln. in 95% ethyl alcohol)	0.3 cc.
	Erythrosin (satd. soln. in 95% ethyl alcohol)	2.0 cc.
12.	Methylene Blue[b] (1% soln. in 95% ethyl alcohol)	0.3 cc.
	Orange G[b] (satd. soln. in 95% ethyl alcohol)	1.0 cc.

[a] National Aniline & Chem. Co.
[b] This stain showed a green precipitate within a few minutes after mixing. Good results were obtained with this combination, but due to the formation of this precipitate it was discarded.

Giemsa's Stain, being a solution in equal parts of methyl alcohol and glycerin, required somewhat different handling than the others. Only half of the solvent was volatile so when the solutiön was ignited the methyl alcohol burned off readily but the glycerin began to bubble and "splutter" as soon as the methyl alcohol had burned off. It was found that if the flame was blown out as soon as the glycerin began to bubble, the slide washed and examined, the sections showed good differentiation.

METHOD III

In the investigation of the double stains, it was found that in sections which contained cork in addition to lignified and cellulose tissue, a "triple-color" differentiation could be obtained. This was observed first in the Methylene Blue-Eosin blood stains. The Methylene Blue stained the lignified tissue blue and the cork tissue green, while the Eosin stained the cellulose tissue pink or red. Since the Methylene Blue gave two colors, it seemed that this "triple color" stain could be obtained with any double stain in which Methylene Blue was the primary stain. This was successfully proved by other combinations using Methylene Blue with three other counterstains, Acid Fuchsin, Orange G and Erythrosin.

The double stains which gave this "triple-color" differentiation were the following:

1.	Wright's Stain (standardized formula)	
2.	Jenner's Stain (satd. soln. in 95% ethyl alcohol)	
3.	Wright's Stain (N. A. C. Co., powder, satd. in 95% ethyl alcohol)	
4.	Giemsa's Stain (standardized formula)	
5.	Azure II—Eosin (satd. soln. in 95% ethyl alcohol)	
6.	Methylene Blue (1% in 95% ethyl alcohol)	0.2 cc.
	Acid Fuchsin (0.5% in 95% ethyl alcohol)	1.0 cc.
7.	Methylene Blue (1% in 95% ethyl alcohol)	0.5 cc.
	Erythrosin (satd. in 95% ethyl alcohol)	2.0 cc.
8.	Methylene Blue (1% in 95% ethyl alcohol)	0.3 cc.
	Orange G (satd. in 95% ethyl alcohol)	1.0 cc.

In Giemsa's Stain and in Azure II—Eosin, the primary stain is Azure II, which is not Methylene Blue alone, but a combination of the HCl salts of Methylene Blue and Methylene Azure (Methylene Blue Sulfone).

To date, these methods have been used successfully on cross-sections of the leaves of dicotyledonous plants: *Jasimum primulinum*, *Cinnamomum camphora* and *Mentha citrata*, and a monocotyledonous plant, *Holcus halepensis;* on cross-and longitudinal-sections of the stems of dicotyledonous plants, *Crotalaria retusa* and *Cracca virginiana* and cross-sections of the stems of a monocotyledon, *Holcus halepensis;* on cross- and longitudinal-sections of the roots of two dicotyledonous plants, Southern smilax and *Cracca virginiana;* and cross- and longitudinal-sections of the root of a monocotyledon, *Zingiber officinale;* and on the germinating embryo of the avocado (*Persea americana*).

In more than 200 slides stained by this method there has been observed no apparent damage to the cells or their contents. In the leaf sections the

chloroplasts were apparently unchanged by the staining process. In a series of more than 30 slides of the root of smilax, the differentiation was very good and the contents of the storage parenchyma cells, such as starch grains and calcium oxalate rosettes, were apparently unchanged by the staining process.

Of twenty-seven alcohol-soluble stains used, only one—Sudan III—proved entirely unsuccessful. Those which were called "poor" in the discussion of Method I were applied with a fair degree of success on a few slides. However, they required special handling which increased the time consumed to such an extent as to render the stain of little practical value. For example, Hæmatin was used as a primary stain on several slides, but it had to be flamed on the slide several successive times without washing after application, and then counterstained with a rapidly acting stain from Group I of Table I. Likewise, Methyl Red was used as a counterstain by applying a primary stain such as Methylene Blue several times, then using the Methyl Red and flaming it on the slide several times. For this reason those stains called "poor" should be entirely discarded due to the fact that they require too much time, too much handling and they do not give as good results as others which are easily applied.

A possible disadvantage of this method was seen in the fact that each slide must be handled individually throughout the entire staining process. However, in the usual methods of staining, the slides must be given individual handling at some stage of the process even when they are stained in groups.

Another possible disadvantage of this method which was apparent was that the staining solutions were used only once. The primary stain must be flamed and occasionally the counterstain also. The washing was brief, so the counterstain was rapidly discolored by the primary stain which was partially retracted. However, if the stains were applied by means of a medicine dropper, only two to four drops were used. The concentration of the staining solutions need never be greater than 1 per cent and in most cases could probably be considerably less. For example, Methylene Blue was equally effective in 0.1 per cent and in 1 per cent solution. Malachite Green was equally effective in 0.1 per cent, 1 per cent and in 7.5 per cent (saturated) solution.

SUMMARY

This paper is presented as a preliminary report, and it is hoped that these methods (although two methods were described separately, both are dependent upon the same principle, namely, flaming the primary stain) may prove of value to other workers in the field, and also that additional information may be brought to light by further investigation.

The authors feel that the great saving of time effected by this method should attract the attention of others interested in perfecting rapid methods of differential botanical staining.

At this time the results of these investigations are applicable only to temporary mounts. The first slides stained by this method were mounted in Canada balsam October 25, 1938. Sufficient time has not elapsed for confirmation of the permanency of the stains.

It is the intention of the authors to conduct further research on this problem; to attempt to establish a more standardized technique; to investigate further the concentration of the staining solution necessary for effective differentiation, as well as to apply other stains and combinations of stains to this technique.

Announcement of a Study to Evaluate Original Serologic Tests for Syphilis

More than five years ago the Committee on Evaluation of Seriodiagnostic Tests for Syphilis, in coöperation with the United States Public Health Service, conducted a study to evaluate original serologic tests for syphilis or modifications thereof in the United States. The results of this study were published shortly after the investigation was completed.[1]

Consideration is now being given by the Committee to the organization of a second evaluation study of original serologic tests for syphilis or modifications thereof within the next year. The second evaluation will be conducted utilizing methods comparable to those employed in the first study.[2]

Serologists who have an original serologic test for syphilis or an original modification thereof and who desire to participate in the second evaluation study should submit their applications not later than October 1, 1940. The applications must be accompanied by a complete description of the technic of the author's serologic test or modification. All correspondence should be directed to the Surgeon General, United States Public Health Service, Washington, D. C.

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[1] *Ven. Dis. Inform.*, Washington, 16 (1935), 189. *J. Am. Med. Assoc.*, Chicago, 104 (June 8, 1935), 2083.

[2] *J. Am. Med. Assoc.*, Chicago, 103 (Dec. 1, 1934), 1705.

Particle Size Studies*

I. The Andreasen-Berg Pipette and the Grain Size Distribution of Barium Sulfate U. S. P.

By John J. Corcoran† and Sister Mary Etheldreda, F.S.S.J.‡

INTRODUCTION

The importance of proper grain size for such pharmaceutical chemicals as the basic bismuth salts, barium sulfate, calomel, kaolin and zinc oxide is now well recognized. The Pharmacœpia (1), under Barium Sulfate describes a test for bulkiness of powder. Eder (2) has recently reviewed and discussed the significance of the grain size of particles; evaluated the sedimentation methods and suggested a microscopic method for Barium Sulfate. Mutzenberg (3) has described a centrifuge method. The National Formulary Revision Committee (4) has adapted a sedimentation test for particle size for Magma of Colloidal Kaolin. Danckwortt (5), using the Andreasen-Berg (6) pipette has reported on a number of commercial German barium sulfate preparations. More recently, in this country, Loomis (7) has used this method for the study of a number of ceramic kaolins. The authors have used this method to determine the grain size distribution in two samples of Barium Sulfate, U. S. P. The pharmacopial test for bulkiness (1) does not measure this factor.

EXPERIMENTAL

Apparatus.—The apparatus consists of a glass cylinder about 6 cm. in diameter having a capacity of approximately 550 ml. when filled to the upper mark on the scale. It is provided with a ground glass stopper through which passes the stem of a pipette, which extends 20 cm. beneath the surface of the suspension and about 4 cm. from the bottom, the tip being at the level of the zero mark on the scale while the upper surface of the suspension is at the 20 cm. mark. The pipette has a capacity of 10 ml., and it is provided with a three way stop-cock and spout for drainage.

Method.—A one per cent suspension was used in the determination. This was prepared as follows: 5.5 Gm. of barium sulfate was levigated with 100 ml. of water containing 50 mg. of barium chloride and 50 mg. of acacia, transferred to the cylinder and diluted to the 20 cm. mark with distilled water. With the stopper of the pipette in place, the mixture was shaken with an end over end motion, for 5 min. The exact time was noted and samples were withdrawn at intervals as follows: 5, 15, 30, 60, 120, 240 and 360 min. The samples were transferred to tared, shallow dishes and evaporated to constant weight at 100° C. The number of mg. of residue gives the percentage of material in the sample.

The grain size of the particles is calculated from Stokes Law as follows:

$$r = \sqrt{\frac{9nh}{2(d_1 - d_2)gt}} \qquad (1)$$

where r is the radius of a spherical particle in cm.
n is the viscosity of the water in poises, 0.00894
d_1 is the density of the barium sulfate, 4.50
d_2 is the density of the water, 0.99707
g is the gravitational constant, 980.267
t is the time in seconds from the start of test
h is the distance (cm.) between liquid surface and pipette when liquid is drawn

Simplified, this becomes:

$$r \text{ equals } 0.003416 \sqrt{\frac{h}{t}} \qquad (2)$$

For the purposes of the test, this was further simplified as follows, where r is in microns and t is in minutes:

$$r \text{ equals } 4.41 \sqrt{\frac{h}{t}} \qquad (3)$$

The results are tabulated in the following tables:

Table I.—Grain Size Distribution of Particles of Barium Sulfate, U. S. P. Manufacturer "A"

Time 10:15	Sample No.	t (Min.)	h (Cm.)	r (Micron)	Dia. ($2r$)	% Finer Than Size
10:20	1	5	20.0	8.82	17.64	89.2
10:30	2	15	19.7	5.38	10.75	87.2
10:45	3	30	19.4	3.54	7.08	85.0
11:15	4	60	19.0	2.46	4.92	83.2
12:15	5	120	18.7	1.71	3.42	68.2
2:15	6	240	18.4	1.19	2.38	20.0
4:15	7	360	18.0	1.05	2.10	2.8

Table II.—Comparison of the Grain Size Distribution of Two Samples of Barium Sulfate, U. S. P.

Diameter	17.64	10.76	7.08	4.92	3.42	2.38	2.10
Grain size ($1.612r$)	14.21	8.67	5.70	3.96	2.75	1.91	1.41

Table III.—Percentage by Weight Finer Than Size

Mfg. "A"	89.2	87.2	85.0	83.2	68.2	20.0	2.80
Mfg. "B"	87.0	85.4	84.8	78.0	45.2	41	33.4

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SUMMARY

1. A method for the determination of grain size distribution of pharmaceutical chemicals has been described.

2. The grain size distribution in two samples of Barium Sulfate have been determined.

CONCLUSIONS

It would seem that a test of this type, incorporating a limit of the per cent of large particles and a requirement for a definite per cent of small particles would be superior to the present pharmacopial test for bulkiness of powder.

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Syrup of Cranberry, A New Pharmaceutical Vehicle

*By J. A. Lubitz, C. R. Fellers and J. A. Clague**

INTRODUCTION

Any attempt to minimize a patient's discomfort and resistance to unpleasant medications is a worthy one. The increase in the number and use of efficient vehicles at the physicians service will eventually neutralize present objections to ill-tasting medicines. The trend to the use of true fruit flavors in pharmaceutical vehicles is a meritorious one. The popularity of Syrup of Cherry, N. F. VI, well illustrates this trend as does the investigation conducted by Mason (1) on grapefruit syrup and Fantus and Dyniewicz (2) on pineapple syrup.

Because of its national distribution, low cost, attractive color and pleasant flavor, the cranberry, *Vaccinium macrocarpum*, in the authors' estimation, warrants attention as the source of a potential pharmaceutical vehicle.

REVIEW OF LITERATURE

Production.—The cranberry is grown principally in Massachusetts, New Jersey, Wisconsin, Oregon and Washington. Its active marketing season extends from September to January, though cranberries are often available during winter months. The annual crop is about 50,000,000 pounds. The retail selling price is variable and depends on the total production, but is usually from $6.00 to $10.00 a barrel of 100 pounds. At retail the cost varies from 7 to 15 cents a pound.

Cranberry Syrup.—A cranberry syrup for beverage purposes was produced commercially in this country in 1895 under the name of "Ruby Phosphate." This was produced at Wareham, Massachusetts, by B. P. Waters and R. C. Randall, local pharmacists, and enjoyed a moderate sale.

Constituents.—The pure cold-pressed juice according to Rice, Fellers and Clague (3) has the following percentage composition: soluble solids 6.7, pectin (alcohol precipitate) 0.13, titrable acidity calculated as citric acid 2.60, ash 0.16 and astringency (tannin) 0.5. The p_H of this juice is 2.4, and the specific gravity is 1.058.

Isham (4) determined the nature of the acids in cranberries. The average amounts of the acids in the Early Black Variety expressed as per cent are: citric 1.1, quinic 1.0, malic 0.26 and benzoic 0.065. Nealy (5) has recently reported the presence of ursolic acid from cranberries. This substance has been used in medicine to a limited extent.

Morse (6) found the percentage composition of the ash to be: potassium oxide 0.068, sodium oxide 0.003, calcium oxide 0.018, magnesium oxide 0.009, phosphorus pentoxide 0.019, sulfur 0.005, chlorine 0.004, iron 0.00022 and manganese 0.00057. Rice, Fellers and Clague (3) found 0.00036 per cent of total iron in Early Black cranberries. The copper content varies widely, but Massachusetts cranberries contain approximately five parts per million. Isham and Fellers

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(7) found that fresh cranberries are a good source of vitamin C with 70–100 International units per ounce. The vitamin A content is from four to seven International units per ounce. Vitamins B, G and D are only in negligible amounts, if at all, in cranberries.

The anthocyan pigment responsible for the red color of cranberry juice is idæin, according to Willstatter (8), who isolated it from the German wild cranberry, *Vaccinium vitis idaea*. Tin and to a lesser extent iron containers and utensils discolor cranberry juice and darken the color first to purple and then to a brownish black. Nickel and copper also cause a slight darkening of the juice. Aluminum has no observable effect on the pigments. Long storage in flint glass bottles in the light will gradually cause the color to fade with the formation of a dark precipitate.

EXPERIMENTAL WORK

Method of Preparation.—Heat extraction of cranberry juice, though giving a higher yield, is impractical for pharmaceutical use as the pectin extracted would cause the finished syrup to jell. This could be obviated, of course, through the use of a pectin-destroying enzyme such as the commercial preparation known as Pectinol (Rohm and Haas), but the time and expense to the retail pharmacist does not warrant the use of this method. Therefore, only cold extraction methods were used in the investigation.

There were two procedures followed in compounding a cranberry syrup. In the first method 900 Gm. of cranberries were ground to a medium fineness in a food chopper, allowed to stand for one-half hour, strained through four layers of cheese cloth and filtered through coarse filter paper. The yield was 450 cc. of juice (clear and sparkling). The juice was then brought to a boil, removed from the flame and 850 Gm. of sucrose were dissolved in it. When cool the surface scum was removed, and the resulting syrup measured 950 cc.

The second method tried was similar to that given for Syrup of Cherry in the National Formulary, sixth edition. Cranberries were crushed in a food chopper, one-tenth per cent benzoic acid was added and the mixture was allowed to stand at room temperature for two days. Then the juice was expressed through four layers of cheese cloth and filtered through filter paper. The filtrate was brought to a boil, and 850 Gm. of sucrose were added to each 450 cc. of the juice. This was allowed to cool slightly, the surface scum was removed, and 20 cc. of alcohol were added to each liter of syrup.

Syrup of Cranberry prepared by the first method possessed a beautiful ruby color, the characteristic delicious cranberry flavor and a mild, pleasant aroma. This syrup when mixed with one-half its volume of alcohol showed no turbidity, indicating the absence of pectin and compatibility with alcoholic medicinal preparations. It has retained its clarity, flavor and color for over three months, the storage being in a well-lighted (sun) cabinet at room temperature.

On cooling, the cranberry syrup made by the second method, changed into a semi-solid jell. As stated previously, jelling could be avoided by removal of pectin from the juice by means of Pectinol. The first method for preparing cranberry syrup is therefore preferable and this is the syrup referred to in this paper. The jelling of syrup manufactured by the second method was thought to be due to extraction of pectin attributed to the acidity of the juice and the long maceration period.

Organoleptic Tests.—The cranberry syrup was compared with Syrup of Cherry, N. F. VI; Syrup of Raspberry, N. F. VI; Syrup of Citric Acid, U. S. P. XI; Syrup of Orange, U. S. P. XI; and Simple Syrup, U. S. P. XI.

Table I gives the results of taste tests made on the six syrups.

Table I.—Relative Palatability of Plain Flavoring Syrups—Score Sheet

Sampler	Cherry	Citric Acid	Cranberry	Simple	Raspberry	Orange
1	5	4	3	6	2	1
2	4	5	1	6	3	2
3	2	3	4	6	1	5
4	3	5	1	6	4	2
5	4	6	5	3	1	2
6	3	5	6	4	1	2
7	1	6	3	5	2	4
8	5	3	1	6	2	4
9	4	3	2	6	1	5
Score[a]	31	40	26	48	17	27

[a] Points are given to each syrup on basis of preference, *i. e.*, first choice 1 point, second choice 2 points and so on. These points are added to give a "score" so that a basis for comparison may be obtained; the lowest score being the favored syrup, the highest score being the least palatable syrup.

The palatability preference was as follows: Syrup Raspberry, first; Syrup of Cranberry, second; Syrup Orange, third; Syrup Cherry, fourth; Syrup of Citric Acid, fifth; and Simple Syrup, sixth.

Disguising Properties.—The masking properties of these syrup vehicles were tried using them as carriers for the following drugs: potassium iodide, chloral hydrate, potassium acetate, ammonium chloride and sodium citrate.

These medicinal preparations were compounded in 60 cc. amounts. The quantities of the drugs used were: potassium iodide, 5 Gm.; chloral hydrate, 5 Gm.; ammonium chloride, 5 Gm.; potassium acetate, 15 Gm.; sodium citrate, 7.5 Gm.

Potassium acetate and sodium citrate, due, no doubt, to their alkalinity, caused the red color of

cranberry syrup to change to a not unattractive light molasses color.

According to Table II, cranberry syrup ranked fifth in score as a vehicle for potassium iodide; tied in first place with Syrup of Citric Acid as a vehicle for chloral hydrate. As a masking agent for potassium acetate, cranberry again tied with Syrup of Citric Acid, this time in second place. As a vehicle for ammonium chloride it ranked in first place; and as a vehicle for sodium citrate, it ranked last.

Table II.—Relative Palatability of Drugs in Several Flavoring Syrups—Score Sheet

Drug	Sampler	Cherry	Citric Acid	Cranberry	Simple	Raspberry	Orange
Potassium iodide	1	1	6	6	2	3	6
	2	6	6	6	6	1	2
	3	2	6	5	1	3	4
	4	2	6	5	1	3	4
		11	24[b]	22[b]	10	10	16
Chloral hydrate	5	3	2	1	6	6	4
	6	2	3	4	6	5	1
	7	6	2	1	1	5	4
	8	6	2	3	3	5	4
		17	9	9	16	21	13
Potassium acetate	9	1[a]	2	6	6	6	1[a]
	10	6	2	1	4	5	3
	11	6	4	2	5	1	3
	12	4	3	2	6	4	3
		17	11	11	21	16	10
Ammonium chloride	13	2	1[a]	1[a]	6	6	2
	14	1	4	2	3	6	6
	15	5	4	3[a]	3[a]	1	2
	16	1	3	2	4	6	5
		9	12	8	16	19	15
Sodium citrate	17	3	2	5	1	6	4
	18	3	2	6	5	4	1
	19	2	5	6	3	1	4
	20	2	3	4	6	1	4
		10	12	21	15	12	13

[a] Both rated in the same place. See Table I for method of scoring.
[b] Signifies that preparation was highly objectionable and counted as 6.

SUMMARY AND CONCLUSIONS

Two methods for the preparation of cranberry syrup were tried. The method recommended for a pharmaceutical Syrup of Cranberry is one in which the juice is expressed cold, filtered, boiled and sweetened.

Potassium acetate, sodium citrate and alkalies caused a color change in this syrup.

The estimated average cost of syrup of cranberry, excluding labor, is not over 25 cents per 1000 cc.

Syrup of cranberry is an efficient vehicle for chloral hydrate, potassium acetate and ammonium chloride. It is compatible with most alcoholic preparations.

Tin, iron, nickel and copper utensils and containers cause darkening of the syrup.

Cranberry syrup is stable in color and flavor toward sunlight under ordinary storage conditions. The syrup will not spoil readily due to high sugar content, acidity and mildly bacteriostatic substances such as benzoic acid present in the cranberries.

Storage is recommended in well-filled, tightly-stoppered, amber glass containers.

It is suggested that further research on Syrup of Cranberry be carried out to ascertain its value as a carrier for other drugs.

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Ointment of Mercuric Nitrate*

By Rudolph A. Kuever† and Carl B. Burnside‡

INTRODUCTION

Ointment of Mercuric Nitrate has long been in use as an antiseptic preparation and is widely employed to-day as an antiseptic application in various skin diseases such as impetigo, sycosis, ring worm and in certain forms of chronic eczema. It was originally derived from an ointment of lard and nitric acid, called Alyon's Ointment after the man who first prepared it. The Ointment of Nitric Acid of the former Edinburgh and Dublin Pharmacopœias was of this character

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(1). Ointment of Mercuric Nitrate was official in U. S. P. IX and is now official in the N. F. VI. The first preparation under this name appeared in the London Pharmacopœia, of 1650. It contained no mercury however, but was made from a number of mineral ingredients including coral, quartz, sea shells, marble, white lead and tragacanth incorporated with lard, suet and chicken fat. The first ointment of record containing mercuric nitrate was in the Edinburgh Pharmacopœia, of 1722. A variety of ingredients and processes have appeared in the many revisions down to the present time (2).

It has been proven that Ointment of Mercuric Nitrate, N. F. VI, has the highest antiseptic potency of any U. S. P. or N. F. ointment which is now official (3). It is also well known that Ointment of Mercuric Nitrate, N. F. VI, has very poor keeping qualities. After a short time the ointment changes from citrine yellow to a brown color and liberates pungent fumes due to certain oxides of nitrogen. After a long time the ointment acquires a dirty greenish and mottled color, and becomes very hard. In this condition it can only be used when mixed with additional lard. The ointment is very sticky. It is evident that the creation of Ointment of Mercuric Nitrate, *i. e.*, the suspending of substantial quantities of the electrolyte, mercuric nitrate and an excess of nitric acid in lard, was the work of a novice. In a relatively short time the nitric acid is entirely decomposed and therefore can serve no purpose in holding the mercuric nitrate in solution or in suspension. The lard likewise undergoes a change as is evidenced by an alteration both in color and physical properties. The previous explanation that the citrine color of freshly prepared ointment of mercuric nitrate is due to the formation of elaidin from olein can no longer be accepted since the same shade of yellow color is obtained when the ointment is made with petrolatum as a base.

EXPERIMENTAL

With the foregoing facts in mind the following work was undertaken in an attempt to prepare a stable form of ointment of mercuric nitrate. This was accomplished by dispersing an aqueous mercuric nitrate phase in a proposed cholesterol-petrolatum base capable of holding large quantities of aqueous solutions or suspensions in a rather permanent form (4).

After some experimentation the following formula for ointment of mercuric nitrate was developed.

Mercuric nitrate	11.34 Gm.
Nitric acid	1.35 Gm.
Distilled water	32.31 Gm.
White wax	5.00 Gm.
Cholesterol	1.50 Gm.
White petrolatum	48.50 Gm.
To make	100.00 Gm.

Mix 11.34 Gm. of finely powdered mercuric nitrate with 1 cc. of water, preferably in a mortar, and add 1.35 Gm. of nitric acid, accurately weighed. Triturate in the mortar until solution is effected and add 31.31 Gm. of water, slowly and with constant stirring.

Melt the white petrolatum, the cholesterol finely powdered and the white wax in a suitable dish. Continue the heat until the temperature of the mixture is raised to 80° C. and the cholesterol has completely dissolved. Stir the mixture until it congeals. By trituration, slowly incorporate the aqueous solution of mercuric nitrate into the ointment base. Care should be taken to avoid contact with metallic instruments or containers.

An ointment made according to the above formula has the same mercuric nitrate content as the one described in N. F. VI, *i. e.*, 11.34 Gm. per 100 Gm.

When this ointment is first prepared it is snow white, gradually, however, assuming a beautiful light yellow color which it retains permanently. It has an excellent consistency, appreciably enhanced by the five per cent of white wax it contains. After a period of several months the ointment shows no signs of deterioration. It holds its

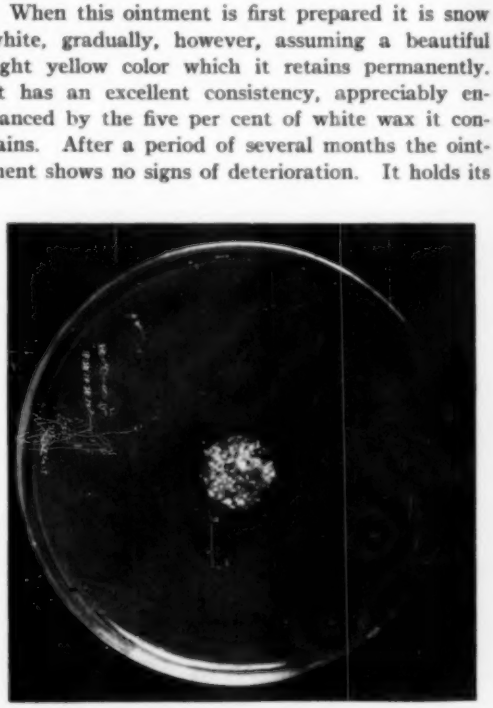

Fig. 1.—Ointment of Mercuric Nitrate, N. F. VI. Test Organism: *Staphylococcus aureus* of 24 hour broth culture at 37° C. Width of Zone: 4 mm. Mercury Coefficient: 0.61.

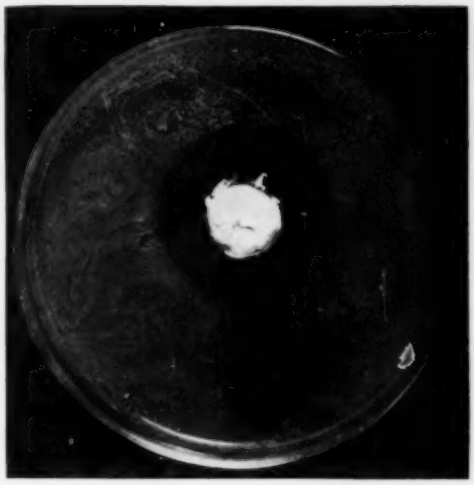

Fig. 2.—Proposed Ointment of Mercuric Nitrate. Test Organism: *Staphylococcus aureus* of 24 hour broth culture at 37° C. Method: Agar-cup. Period of Incubation: 48 hours at 37° C. Width of Zone: 13 mm. Mercury Coefficient: 2.0.

aqueous phase completely and retains its light yellow color and its original antiseptic potency.

The proposed ointment was tested for antiseptic potency in comparison with the N. F. VI ointment using *Staphylococcus aureus* as the test organism. The results were quite satisfactory as the accompanying photographs show.

MERCURY COEFFICIENT

Reddish and Wales (3), in 1929, experimenting with Ointment of Ammoniated Mercury U. S. P. X, obtained a zone 5 mm. wide using *Staphylococcus aureus* as the test organism on plain agar medium. Bryan (5) in 1936, made the same ointment with lanolin as a base, and obtained an inhibited zone having a width of 8 mm. It was this figure he used to compute the mercury coefficient of forty different ointments. Li and Kuever (4) tested U. S. P. Ointment of Ammoniated Mercury and obtained an inhibited zone of 6.5 mm. To find the mercury coefficient of an ointment, using U. S. P. XI Ointment of Ammoniated Mercury as a standard, divide the width of the inhibited zone of the ointment tested by 6.5.

Name	N. F. Ointment	Proposed Ointment
Organism	*Staphlococcus aureus*	*Staphlococcus aureus*
Method	Agar-cup	Agar-cup
Zone	4 mm.	13 mm.
Mercury coeff.	0.61	2.00

SUMMARY

The proposed Ointment of Mercuric Nitrate is more than three times as potent as Ointment of Mercuric Nitrate N. F. VI, when tested against *Staphylococcus aureus*.

The proposed Ointment of Mercuric Nitrate is a relatively permanent preparation which will retain its antiseptic potency and its original physical properties.

The proposed Ointment of Mercuric Nitrate is noticeably superior in consistency and texture when compared with the ointment made according to the process given in the N. F. VI.

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A Rapid Procedure for the Manufacture of Saponated Solution of Cresol*

By Lawell F. Martin† and William A. Proul‡

Saponated Solution of Cresol, formerly known as Compound Solution of Cresol, did not make its appearance in the United States Pharmacopœia until the Eighth Revision. However its chief constituent, cresol, had been in use as a germicide for many years prior to the issuance of the Eighth Revision of the Pharmacopœia.

The fact that cresol is readily miscible with soap solution has led to its popularity in detergents. It is natural enough then, that Saponated Solution of Cresol should have found its way into foreign Pharmacopœias, such as the British, German and others. Due, no doubt, to its relatively high

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phenol coefficient, this widely employed preparation is retained in the Eleventh Revision of the United States Pharmacopœia. Little or no decided change has been made in this monograph since its initial appearance, although many changes have been proposed from time to time as difficulties were encountered in the manufacture of the solution. Kaufman and Lee (1) have pointed out some of the objectionable features of the official formula and procedure, namely; the variability of time usually required for its preparation; the use of two alkalies, when one should suffice; and the probable loss of cresol due, no doubt, to the prolonged heating which is often necessary in order to bring about complete reaction between the reacting substances.

Table I.—Ingredients of Saponated Solution of Cresol, U. S. P.

United States Pharmacopœia Constituents	VIII	IX	X	XI
Cresol	500 Gm.	500 Gm.	500 cc.	500 cc.
Linseed oil	350 Gm.	300 Gm.	350 cc.	350 cc.
Potassium hydroxide (85%)	80 Gm.	80 Gm.	...	14.52 Gm.
Potassium hydroxide dekanormal sol.	...	...	22 cc.	....
Sodium hydroxide (95%)	...	...	...	37.05 Gm.
Sodium hydroxide dekanormal sol.	...	...	88 cc.	....
Alcohol	...	30 mils	...	....
Water, *q. s.* to make	1000 Gm.	1000 Gm.	1000 cc.	....
Distilled water, *q. s.* to make	...	...	...	1000 cc.

EXPERIMENTAL

Investigation of this preparation was undertaken to eliminate these objectionable factors by combining, if possible an already prepared soap, such as sodium stearate or sodium oleate directly with the cresol, rather than manufacturing the soap within the solution.

In substituting an already prepared constituent in place of one manufactured in a preparation, the amount to be used is generally based upon the quantity produced between the reacting agents. The equivalent amount of sodium stearate or sodium oleate was therefore substituted for the alkalies and linseed oil designated in the pharmacopœial formula. As a result of this combination, gelatinization occurred in almost every instance. Contrary to the report of Kaufman and Lee, (1) gelatinization occurred regardless of the quality of the cresol used.[1] It was decided, if possible, to trace the cause of gelatinization, since the question of the quality of cresol was apparently not involved. It was noted that preparations made as outlined on the basis of the pharmacopœial formula showed an excess of soap in the finished product, which led to the belief that too much soap was being used. Hilton's formula (2) which has proved to be a very satisfactory one, was finally decided upon as the basis of calculation of the amount of soap to use. Hilton's formula is as follows:

Cresol	500 Gm.
Oleic acid	226 Gm.
Sodium hydroxide	35 Gm.
Water, *q. s. ad*	
to make	1000 mils

From this it was calculated that 240 Gm. of sodium oleate or sodium stearate were required to replace the amount of oleic acid and sodium hydroxide in the above formula. This was considerably less than the amount calculated upon the basis of the official formula. The revised formula is as follows:

Cresol	500 cc.
Sodium oleate or Sodium stearate	240 Gm.
Distilled water, *q. s. ad*	
to make	1000 cc.

Dissolve the soap in the cresol, to which about 200 cc. of distilled water have been added. Heat the mixture, with constant stirring, to about 65° C., and maintain this temperature until solution is effected. Cool the liquid, add sufficient distilled water to make the product measure 1000 cc. and mix well.

DISCUSSION

Approximately five minutes were required to prepare the solution using either sodium stearate or sodium oleate. Gelatinization did not occur in any of the solutions made by this formula. The initial appearance of the solution is practically the same regardless of which soap is used. The solution made with the sodium oleate, however, presents an extremely slight precipitate which forms after several hours standing, but no increase is noted upon further standing. The precipitate does not appear objectionable, as it is so very slight and can be easily dispersed by agitating the solution. The initial color of the solutions (light amber) prepared as above is much lighter than that of the official preparation, probably because of the

[1] U. S. P. Cresol (Baker's), fresh stock.

fact that much less heat is required in its manufacture. They do become darker, however, upon standing unless well protected from light.

Objections have been raised relative to the dark color of the pharmacopœial solution. As it usually requires an indefinite interval, generally an hour, to prepare the official solution by heating at 70° C., this suggested the probable cause of the dark color of the finished product. Experiments were carried out, therefore, to ascertain to what extent, if any, prolonged heating had upon the color. Cresol, U. S. P. (fresh stock) whose initial color is very light amber, was used as the control. It, along with the Saponated Solution of Cresol made in accordance with the revised formula, was heated at 70° C., for one hour. At the end of this time, both the cresol and the Saponated Solution of Cresol had acquired a very deep amber color. This color appeared to be about the same shade as that of the official preparation. These heating experiments confirm the report of O'Day and Jones (3) who investigated the Saponated Solution of Cresol, employing both the hot and cold method of manufacture. Ordinary light also affects the color of the solutions containing cresol. Even in dilutions of 1:250, the solutions become decidedly pink, and after having stood several days, the color deepens. The present revision of the pharmacopœia designates that cresol be preserved in well-closed containers and protected from light, yet, no such provision is made for the preparation of the Saponated Solution of Cresol with respect to the protection from light.

Prolonged heating also appears to affect the content of cresol as is shown by analyses carried out according to Handke's (4) method. The United States Pharmacopœia X Compound Solution of Cresol assayed approximately forty-four per cent cresol, the present official Saponated Solution of Cresol assayed approximately forty-six per cent cresol, while the Saponated Solution of Cresol made according to the revised formula assayed approximately forty-nine per cent cresol.

The following specific gravities of the cresol solutions were determined with the Mohr-Westphal Balance: U. S. P. Compound Solution of Cresol 1.0356; U. S. P. Saponated Solution of Cresol 1.0350; and Saponated Solution of Cresol (revised formula) 1.0272. These figures show how closely the specific gravity of the solution prepared in accordance to the revised formula compares with the specific gravities of the U. S. P. X and U. S. P. XI preparations.

CONCLUSIONS

1. Saponated Solution of Cresol can be easily and speedily prepared by using either sodium stearate or sodium oleate in direct combination with the cresol.

2. Very little heat is required in the manufacture using the revised formula, thus assuring a relatively high per cent of cresol in the finished product.

3. Prolonged heating affects the color of the solution. The preparation made according to the revised formula presents a much lighter-colored solution than the official product.

4. Ordinary light also affects the color of the finished preparation, therefore, provision should be made to protect preparations containing cresol from light during storage.

5. Gelatinization does not occur in the Saponated Solution of Cresol when made according to the revised formula, regardless of the grade of cresol used. While the cause of gelatinization is apparently not entirely solved, it is suggested that possibly too much soap is produced and too much heating is required in the official formula.

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NOTICE

Please send in immediately completed manuscripts for papers read at the Richmond meeting.

Psychic Medicine

*By Charles Whitebread**

Psychic, or mind, medicine is a general term applied to methods of treating disease through mental impressions. States of mind are induced, or mental qualities stimulated to such a degree as to modify physiological function, and through such modified function even to produce tissue change. Confidence, hope, faith are aroused, the imagination excited, the attention fixed by suggestion or individual effort, or certain peculiar mental conditions induced which are known by such names as mind healing, faith cure, hypnotism, etc.

These psychic conditions may be established by sensory impressions, as by music, visual objects to fix the attention, eyestrain to modify consciousness, by manipulation, by personal magnetism of the mind doctor or by mental effort on the part of the patient.

Fig. 1.—Egyptian Harp.

From a wall painting in a tomb at Thebes. This harp probably represents a larger and more elaborate form of the musical instrument used by David for the relief of Saul. Courtesy of U. S. National Museum.

MUSIC

The use of instrumental music in the treatment of disease is of very ancient origin. David is said to have cured Saul of an attack of mania by playing on the harp in his presence:

"But the spirit of the Lord departed from Saul, and an evil spirit from the Lord troubled him. And it came to pass, when the evil spirit from God was upon Saul, that David took an harp and played with his hand; so Saul was refreshed and was well, and the evil spirit departed from him." Samuel, xvi 14–23.

Jean Battiste Porta, in the 16th century, advised that musical instruments be made of wood of medicinal plants, and affirmed that the music of these instruments would produce the medicinal effects of the plants.

In later times music was successfully used in the treatment of the delirium of fevers, the plague, gout, poisoned wounds, to mitigate the pain of surgical operations and especially in nervous affections such as hysteria and mania.

Music is beneficial in the management of the insane, and musical entertainments are frequent in modern institutions for their treatment.

ROYAL TOUCH

Royal Touch was a mode of mind healing which is said to have originated with an English king, Edward the Confessor, 1004–1066. It was practiced by succeeding kings and queens of England, with occasional exceptions, down to Queen Anne, 1664–1714. A register of persons touched by King Charles II, from May 1662 to April 1682, gives the number at 92,107. Healing by touch was also practiced by French kings, and it is claimed by some French historians that the custom originated in France. Until the time of Henry VII no particular ceremonies attended the practice of healing by touch. This monarch established a special religious service to be employed at the healings, during which a piece of gold (touchpiece) was presented to the patient, to be worn as an amulet suspended from the neck.

The ceremony of touching for scrofula, or King's Evil, as practiced by Charles II, is described by Evelyn in his memoirs as follows:

"July 6, 1660. His majestie sitting under his state in ye banquetting house, the chirurgeons cause the sick to be brought, or led up to the throne, where they kneeling, ye king strokes their faces or cheeks with both his hands at once, at which time a chaplaine in his formalities says: 'He put his hands upon them and he healed them.' This is said to everyone in particular. When they have all been touched they come up againe in the same order, and the other chaplaine kneeling, and having angel gold strung on white ribbon on his arme, delivers them one by one to his majestie, who puts them about the necks of the touched as they passe, while the first chaplaine repeats, 'That is ye true light who came into ye world.' Then follows an epistle (as at first a gospell) with the liturgy, prayers for the sick; lastly ye blessing; and then the lord chamberlaine and comptroller of the household brings a basin, ewer and towell, for his majestie to wash."

It is claimed now that the cures effected by Royal Touch, which are so often cited as proof of the power

* Associate Curator, Division of Medicine and Public Health, United States National Museum, Washington, D. C.

Fig. 2 —Royal Touch.

A model depicting the laying on of hands for the cure of scrofula, as practiced by European kings and queens. Courtesy of U. S. National Museum.

of faith over disease, or of mind over the body, were produced by very different causes. It is said that it was a part of the duty of the royal physicians and surgeons to select such patients afflicted with scrofula as evinced a tendency toward recovery, and that the touch of the king secured the patient from the mischievous importunities of ancient medical art, so that the efforts of nature to heal were left free and unhampered with the result that the cure of the disease was not retarded by the operation of adverse remedies.

LAYING ON OF HANDS

Valentine Greatrakes, an Irishman, attained great reputation about the middle of the 17th century by his marvelous cures of diseases by simply stroking with the hands. At first he confined his efforts to the cure of scrofula, or King's Evil; subsequently he treated patients with many other diseases, such as apoplexy, convulsions and palsy. In 1666, a physician of Oxford, had the following to say about Mr. Greatrakes' ability:

"I saw him stroke a man for a great and settled paine in his left shoulder, which rendered his arme uselesse: upon his stroking it the pain removed instantly into the end of the Musculus Deltodes: being stroked there, it returned to the shoulder again; thence (upon a second stroking) it flew to the elbow, thence to the wrist, thence to the shoulder again; and thence to the fingers: whence it went out upon his last stroking, so as that he moved his arme vigorously every way." From "The Miraculous Conformist," by Henry Stubbe, Physician.

METAL THERAPY

The curing of disease by the application of metallic substances to the surface of the body, is another method of therapy which falls under the head of mind medicine in most instances, appealing as it does to the credulity and imagination of the patient. It has been advocated from the time of the Middle Ages to the present, principally as a prophylactic and a remedy for distinctly nervous diseases.

A plate of copper, worn as a preventive of cholera and a remedy for facial neuralgia and other nervous

Fig. 3.—Valentine Greatrakes.

A psychic medicine practitioner of Affane, County of Waterford, Ireland, famous for curing by the stroke of his hands. (Published June 1, 1794, by I. Caulfield.) Courtesy of U. S. National Museum.

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Many people in Germany in the first half of the 19th century wore a small tube of mercury suspended from the neck as a protection from the cholera. This practice had its origin in the immunity of workmen in quicksilver mines during an epidemic of cholera.

The alleged healing properties of an old horseshoe is another example of mind medicine. This was considered such a wonderful remedy for cramps, that it was customary to keep a horseshoe at the bedside where it could be reached in case of an attack. The healing effects attributed to the horseshoe were said to be due to a magnetic quality it had acquired by long beating upon stony roads and pavements.

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HYPNOTISM

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An apparatus used for hypnotizing, a so-called mirror hypnotizer, consists of two wooden arms about 9 inches long, in each of which are set circular mirrors, seven on each side. The two arms are made to revolve in opposite directions by means of clock work. Many subjects, particularly those who have been hypnotized before, are susceptible to the dazzling effects of these revolving mirrors.

Another type of hypnotizer is a polished nickel-plated ball mounted on a lead wire, and attached to a headband. The lead wire is bent so that the ball is a little above the level of the eyes of the subject, and slightly within the natural focal distance. Staring at the ball creates eyestrain and fatigue of the ocular muscles, and produces the effect of hypnotism. The effect of this and other types of hypnotizers is usually intensified by "suggestion" on the part of the operator.

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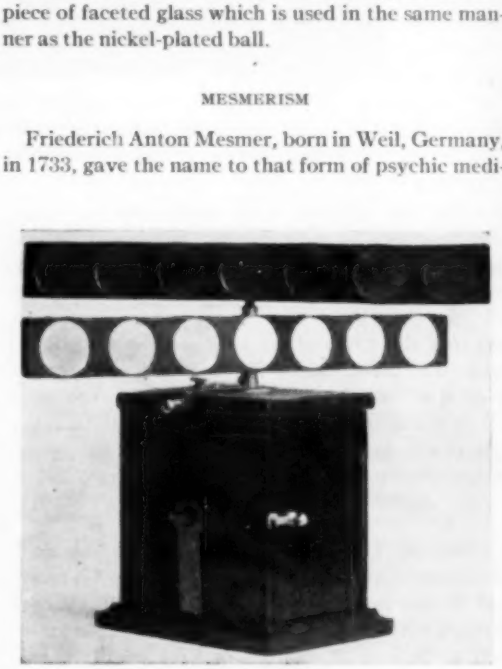

Fig. 4.—Mirror Hypnotizer.

An apparatus for hypnotizing. (Man and Abnormal Man, Arthur MacDonald, p. 189.) Courtesy of U. S. National Museum.

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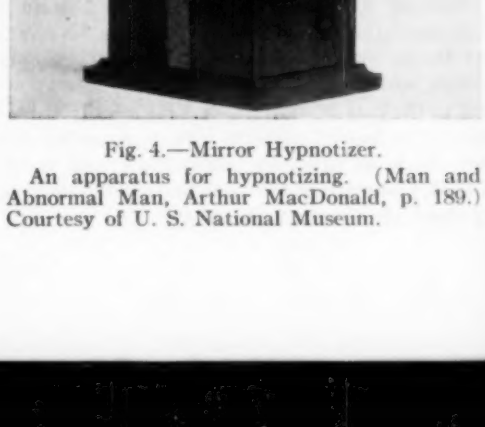

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exhausted. There is still a great need for psychic medicine, and those who select any branch of the healing art as a calling will do well to maintain a tolerant attitude toward this type of medication.

Book Reviews

Practice of Allergy, by WARREN T. VAUGHAN, M.D. Price, $11.50, pages 1082, with 338 illustrations. St. Louis: C. V. Mosby Company, 1939.

This is the newest and most complete book on the subject of allergy. It contains 80 chapters dealing thoroughly with every phase of the subject. Take, for instance, the chapter on diagnosis which is typical of the thoroughness of which each phase of the subject is handled. In this chapter, history taking, testing and evaluation of findings are discussed. Detailed directions are given for carrying out all of the different techniques of testing including the less frequently used diagnostic procedures such as passive transfer, mucous membrane tests, patch tests, leucopenic index, elimination diets and the examination of nasal secretions. The botanical aspects of allergy are unusually well covered. The author has gathered and condensed all existing contributions to this field and has included atmospheric studies up to and including 1938. There are more than 100 photographs of plants, in addition to regional maps, charts and photomicrographs of pollen. There are four chapters on fungi and these are likewise well illustrated. Food allergy is given 138 pages and space is also given to drug, entomogenous, contact and physical allergies. The discussion of the therapy of allergies deals with all of the methods in use and summarizes the procedures used in allergic clinics throughout the country. The last section of the book is devoted to a systematic discussion of the major and minor allergic diseases—asthma, hay fever, migraine, dermatitis, urticaria, gastro-intestinal allergy, epilepsy, arthritis, otologic and ocular symptoms, etc. The pathology, diagnosis, prognosis and such forms of therapy as have not been touched upon in the other chapters are also presented. The closing chapters are a plea for the compilation of continuous life records of all persons, but particularly the allergics.—A. G. D.

The Tools of The Chemist. Their Ancestry and American Evolution, by ERNEST CHILD. 220 pages, 105 illustrations. Reinhold Publishing Corp., 330 West 42nd St., New York, N. Y., 1940. Price $3.50.

This book has to do with the evolution of American chemistry in general and the tools used by the chemist. It represents a thorough documentary research on these subjects. The author has given a brief and interesting summary of the European background of American chemistry and has traced the early history of chemistry in this country. He has also reviewed the evolution of the apparatus and instruments used by chemists during this period.

Part I deals with the pioneers and events in American chemistry. Among the persons mentioned are John Winthrop, Benjamin Rush, Joseph Priestley, Benjamin Silliman and Robere Hare.

Part II deals with the ancestry and development of chemical laboratory apparatus in America. It describes briefly the evolution of the balance, the history of the manufacture of glassware and porcelain, heating apparatus, metal appliances and optical instruments.

Part III deals with early American distributors of chemical laboratory apparatus.

The book is well illustrated, there is a good index and it will be found to be of interest to pharmacists as well as chemists.—A. G. D.

Hydrocarbon Chemistry. Reprinted from Transactions of the Faraday Society, 28, Part 8 (1939), 286 pages (806 to 1092). GURNEY & JACKSON, 98 Great Russell St., London, England. Price, 12s, 6d.

Hydrocarbon chemistry is considered under four main subdivisions. Part I deals with the homogeneous thermal reaction of hydrocarbons. The structure and energies of hydrocarbons are discussed. Several papers are presented on the mechanism of hydrocarbon reactions, particularly from the standpoint of Rice's free radical theory. In Part II, certain catalytic reactions are discussed. Part III deals with the mechanism of technical synthesis and the transformation of hydrocarbons. Papers on the catalytic high-pressure hydrogenation of carbonaceous material and the cyclization of aliphatic hydrocarbons are included. Part IV deals with the polymerization of olefins—dimerization, medium molecular weight polymers and high molecular weight polymers. The book is a notable contribution to our knowledge of hydrocarbons and should prove of interest to all organic chemists.—A. G. D.

Micro-Diffusion Analysis and Volumetric Error. by EDWARD J. CONWAY. 306 pages. Crosby Lockwood & Son, Ltd., London, England, 1939. Price, 25s.

The book is divided into three parts. The first part describes the apparatus and principles of diffusion analysis, and includes a discussion of the factors governing the rate and degree of quantitative transfer of the volatile substances, also a description of the pipettes and burettes used in microvolumetric analysis. The second part gives the directions for carrying out specific determinations, by the use of the micro-diffusion apparatus. The third part is a discussion of error in micro- and

macro-volumetric analysis. Whereas the book is intended primarily for biochemists, the treatment of the subject is such and the inclusion of the chapter on volumetric error makes it of interest to all analysts.—A. G. D.

Industrial Solvents, by IBERT MELLAN. 464 pages. Reinhold Publishing Corp., 330 West 42nd St., New York, N. Y., 1939. Price, $11.

This book is principally a compilation of data from most of the important papers on solvents published in the journal literature and from contributions made by the manufacturers of solvents. The first seven chapters deal with the general phases of the subject and, in the following nine chapters, the individual members of each important class of solvents are described. There are numerous tables and charts. The final chapter deals with graphical expression and interpretation. The book should prove useful to pharmaceutical manufacturers.—A. G. D.

Die exacten Methoden der Mikromassanalyse. Die chemische Analyse. Volume 42. J. MIKA. Edited by W. Böttger. 180 pages. Ferdinand Enke Verlag, Hafenbergftelge 3, Stuttgart, Germany, 1939. Price, paper, 18 marks; bound, 19.60 marks.

The author, who has contributed a number of papers on microvolumetric analysis, emphasizes the technique of microvolumetric analysis in this monograph. The book is divided into a general part of 100 pages and a special part of 70 pages. In the introduction, it is pointed out that an accuracy of 0.1 to 1 per cent is desirable in the determination of amounts of several milligrams of a substance. Because of this, thorough consideration is given to the determination of the equivalence point, and in Chapter II, the indicator correction and the potentiometric and conductometric titrations are discussed. Chapter IV deals with the apparatus used in microvolumetric analysis. In the special part, methods of neutralization, precipitation, complex formation and oxidation-reduction analysis are discussed and some practical applications are given. The binding of the book is bad, but it is believed that the material contained therein will be of value to workers in the field of microanalysis.—A. G. D.

Materia Medica, Pharmacology, Therapeutics and Prescription Writing (Fourth Edition), by WALTER ARTHUR BASTEDO, Ph.M., M.D., Sc.D., F.A.C.P., Consulting Physician, St. Luke's Hospital, New York, St. Vincent's Hospital, Staten Island; President of the United States Pharmacopœial Convention 1930–1940, Member Revision Committee U. S. Pharmacopœia; Formerly Curator of the New York Botanical Garden; Attending Physician, City Hospital, New York; Instructor in Pharmacology, Cornell University. 778 pages, 81 illustrations. Philadelphia and London: W. B. Saunders, 1937. Price, $6.50.

This edition of the book is dedicated to Henry Hurd Rusby, Botanist and Pharmacognosist, and the first teacher in Materia Medica of the author. The latter stated that the book is an adaptation, for the most part, of lectures delivered at Columbia University, and that he has drawn on researches in all the branches of medicine. The book is divided into three parts: The first treats of the constituents of organic drugs, pharmaceutical preparations, weights and measures, active principles, the Pharmacopœia and the National Formulary, and of pharmacologic action in a general way. Part II treats of individual remedies and their actions and Part III deals with prescription writing and is therefore of particular interest to pharmacists. Most of the subject matter is arranged to make it easily accessible to physicians and pharmacists and for students and practitioners of medicine and pharmacy.—E. G. EBERLE

New and Nonofficial Remedies—1940, published by the American Medical Association, Chicago, Illinois. Price, $1.50.

This volume of more than 700 pages contains descriptions of the chemical and physical properties, tests for purity and potency, etc., of the articles which had been accepted by the Council on Pharmacy and Chemistry of the American Medical Association on January 1, 1940. In addition, the preface lists the articles described; this list is followed by the names of the members of the Council, a list of consultants and the official rules of the Council on Pharmacy and Chemistry. The latter include rules for admission of proprietary articles, admissible advertising and names, patents, unscientific and therapeutically useless articles and a statement of policies of firms detrimental to rational therapeutics.—E. G. EBERLE

Law of Drugs and Druggists. A Treatise with Text, Cases, Statutes, Readings and Digests for Schools of Pharmacy, Retail, Wholesale and Manufacturing Druggists by WILLIAM R. ARTHUR, Professor of Law, University of Colorado. Second edition. Publishers: West Publishing Co., St. Paul, Minn., 1940. About 600 pages. Maroon fabricoid. Price, $3.00.

The treatise is designed especially for use as a textbook for schools of pharmacy and a reference book for retail pharmacists, wholesalers and manufacturers. Questions are answered on various phases of narcotic legislation, poisons, patent and proprietary preparations; laws relating to prescriptions and beverages; the position of a druggist in court, his testimony, damages, duty to customers, etc. The authors discuss federal, state and local laws, price fixing, advertising, postal regulation, distribution of samples, relation of employer and employee. There is a comprehensive index, cross index; the book has been thoroughly revised, a list of cases fills twelve pages. Where possible, a case is inserted to bring out the principles involved; in some presentations the laws of drugs are made which have no direct

bearing on the text but do on the law of drugs in states or other connection; more than five pages are given to a glossary. The discussion of Boards of Pharmacy is helpful, that of U. S. Pharmacopœia and National Formulary might have been more extended; however, these standards have the support of the Government and the Pharmaceutical organizations.

An Outline and Manual for Teachers and Students on the Law and Druggists by Homer C. Washburn covers more than 100 pages and a pocket is provided for them in the book. This section deals with all phases of law and their pharmaceutical applications and the page numbers provide a ready means and direction for study.—E. G. EBERLE

Pathogenic Microörganisms, by WILLIAM HALLOCK PARK and ARMA WESSELS WILLIAMS. 11th ed., 1056 pages, 5¾ x 9¼. Philadelphia: Lea & Febiger, 1939. Price, $8.00.

This is the eleventh edition of this well-known work on bacteriology. Part I of the book deals with the general principles of bacteriology, such as classification and general characteristics of micoorganisms, particularly metabolism and variability. Part II deals with practical methods of cultivation and examination of bacteria, the handling of specimens and the use of test animals. Part III treats of infection and immunity. Part IV is devoted to pathogenic microörganisms which are taken up according to their taxonomic classification. Parts V, VI and VII deal with the filterable viruses, bacteriophage, pathogenic yeasts and molds and the pathogenic protozoa. The final chapter is devoted to the applications of microbiology in the bacteriological examination of water, milk, sheilfish, soil and in disinfection and sterilization.—A. G. D.

Industrial Dermatoses. 174 pages, 6⅞ x 10, paper. Chicago: American Medical Association, 1930. Price, $1.00.

This book is a compilation of symposia of diseases of the skin traceable to industrial causes. It treats of diseases contracted by workers in abattoirs, in the leather, oil refining, chemical, photographic and other industries. Of particular interest at this time is the article devoted to diseases contracted in the manufacture of war gases. The book should be of interest and value to the pharmaceutical and chemical manufacturers.—A. G. D.

Fundamentals of Biochemistry, by T. R. PARSONS. 6th ed., xii + 461 pages, 4⅞ x 7¼. Baltimore: William Wood & Co., 1939. Price, $3.00.

The book presents in a continuous manner the study of biochemistry. Its reading does not require any great amount of chemical knowledge. The uses of proteins, purines and the amino acids are discussed. Fats, sterols, carbohydrates, enzymes, pigments and respiratory gases are considered, also osmotic pressure, electrolytes and colloids. At the end of each chapter is a short bibliography of important references. It is believed that the book will be found to be of value to pharmacists who desire to refresh their memories on the subject of biochemistry.—A. G. D.

Textbook of Physiology, by WM. D. ZOETHOUT and W. W. TUTTLE. 7th ed., 743 pages, 6 x 9. St. Louis: C. V. Mosby, 1940. Price, $4.50.

The seventh edition of this well-known textbook differs from the preceding one chiefly in that it contains new material and additional illustrations. It is designed primarily for use in schools of dentistry and pharmacy and normal schools where a book which is not too voluminous is desired.—A. G. D.

Physical Organic Chemistry, by LOUIS P. HAMMETT. x + 404 pages, 5½ x 8. New York: McGraw-Hill, 1940. Price, $4.00.

This book is devoted to a discussion of the application of the methods of physical chemistry to the study of organic chemical reactions. The subjects discussed are the structural chemistry of electrolytes and nonelectrolytes, equilibrium and reaction energies, rates, mechanism and entropy, the displacement reaction and its stereochemistry, quantitative study of acids and bases, enolization, carbonium-ion, carbonyl-addition, and atom and radical reactions.—A. G. D.

Handbook of Chemistry and Physics, Ed. by CHARLES D. HODGMAN. 23rd ed., 2221 pages, 4¾ x 7. Cleveland: Chemical Rubber Publ. Co., 1939. Price, $3.50.

This book is so well known to workers engaged in the chemical and allied industries that it does not require reviewing. Suffice it to say that the present edition is larger and more comprehensive than the preceding one. Among the new tables which it contains, the following are particularly worthy of mention: table index of melting points of organic compounds, table index of boiling points, table of potentials of electrochemical reactions, tables showing free energy of formation of certain chemical substances and a ratio table for bridge calculations.—A. G. D.